

**NATIONAL SPACE BIOMEDICAL RESEARCH
INSTITUTE**

**Annual Scientific and Technical Report
October 1, 2002 – September 30, 2003**



Cooperative Agreement NCC 9-58

with the

**National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas**

September 30, 2003

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL SCIENTIFIC AND TECHNICAL REPORT

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1.0 INTRODUCTION

This report outlines the activities of the National Space Biomedical Research Institute (NSBRI) during FY 2003, the sixth year of the NSBRI's programs. It is prepared in accordance with Cooperative Agreement NCC 9-58 between NASA's Lyndon B. Johnson Space Center (JSC) and the Institute's lead institution, Baylor College of Medicine.

2.0 BACKGROUND

In June 1996, NASA released a Cooperative Agreement Notice (CAN) inviting proposals to establish a National Space Biomedical Research Institute (9-CAN-96-01). This CAN stated that:

The Mission of the Institute will be to lead a National effort for accomplishing the integrated, critical path, biomedical research necessary to support the long term human presence, development, and exploration of space and to enhance life on Earth by applying the resultant advances in human knowledge and technology acquired through living and working in space. The Institute will be the focal point of NASA sponsored space biomedical research.

This statement has not been amended by NASA and remains the mission of the NSBRI.

The Institute was selected by NASA in March 1997 following a two-phase, competitive review of proposals received in response to the CAN. In April 1997, the NSBRI was chartered in the State of Texas as a non-profit corporation. After a 60-day definition period, NASA and the NSBRI signed a Cooperative Agreement (NCC 9-58) and Cooperative Agreement Management Plan (CAMP) with JSC. The Cooperative Agreement is for a five and one-half year base period and three, five-year optional extensions. The first optional extension, lasting until September 30, 2007, is being exercised. The CAMP was revised in June 2003 to fully align it with the Institute's Revised Strategic Plan and NASA's Bioastronautics Strategy, both being approved in 2003.

The NSBRI partners with NASA to develop countermeasures against the deleterious effects of long-duration space flight, and performs fundamental and applied space biomedical research directed toward this goal. This is accomplished by:

- designing, testing and validating effective countermeasures to address the biological and environmental impediments to long-term human space flight;
- defining the molecular, cellular, organ-level, integrated responses and mechanistic relationships that ultimately determine these impediments, where such activity fosters the development of novel countermeasures;
- establishing biomedical support technologies to maximize human performance in space, reduce biomedical hazards to an acceptable level and deliver quality medical care;
- transferring and disseminating the biomedical advances in knowledge and technology acquired through living and working in space to the general benefit of humankind; including the treatment of patients suffering from gravity- and radiation-related conditions on Earth; and

- ensuring open involvement of the scientific community, industry and the public in the Institute's activities and fostering a robust collaboration with NASA, particularly through the JSC.

The NSBRI is governed by a consortium of twelve institutions – Baylor College of Medicine, Brookhaven National Laboratory, Harvard Medical School, The Johns Hopkins University School of Medicine and Applied Physics Laboratory, Massachusetts Institute of Technology, Morehouse School of Medicine, Mount Sinai School of Medicine, Rice University, Texas A&M University, the University of Arkansas for Medical Sciences, the University of Pennsylvania Health System and the University of Washington. The Institute's headquarters are located in Houston at Baylor College of Medicine.

The Institute's initial peer-reviewed research program consisted of eight research teams carrying out 37, three-year projects and four, one-year synergy projects designed to bridge between discipline research team activities and create an appropriate atmosphere for future interdisciplinary research. As a result of two open research announcements in FY 2000, the institute expanded to 12 research teams and 85 research projects during its fourth year. In the fifth and sixth years, the number of research teams was consolidated to 11 and an integrated NSBRI/NASA space medicine program was added, bringing the total number of research projects to 94.

In addition to its research program, the NSBRI has developed vital education, outreach and communications programs that take advantage of the Institute's core research activities. Currently there is funding of research and education projects at 81 institutions across 23 states.

The Institute's management plan is based on the model used by the National Institutes of Health. An independent Board of Scientific Counselors (BSC) is responsible for assuring excellence in the Institute's intramural program through independent, external peer review. An External Advisory Council (EAC) provides advice to Institute management concerning programmatic relevance and effectiveness. The NSBRI also has a User Panel of former and current astronauts and flight surgeons responsible for assuring that the research program focuses on astronaut health and safety. An Industry Forum of representatives from aerospace, biomedical and technology industries assists in developing industry participation in NSBRI and in timely technology transfer.

Primary support for the NSBRI's activities is furnished by NASA through NCC 9-58, although funds to support Institute activities also come from several sources, including the institutions involved in carrying out the NSBRI's programs. Initial annual base funding for the Institute's first two years of operation (FY 1998 and FY 1999) was approximately \$10 million. In FY 2000, base annual funding increased to approximately \$14 million to develop the infrastructure needed to support planned program growth in FY 2001. In support of the expanded research and education programs, the base annual funding increased to approximately \$25 million in FY 2001 and was \$22 million in FY 2002. Budget stability of \$30 million per year for FY 2003-2007 was achieved. This figure is divided into \$29 million for core research, education and administrative costs and \$1 million for core space medicine research. The Institute also supports \$2 to \$3 million of non-core research activities that primarily fund a translational workforce that helps link NSBRI and JSC programs. Approximately 85 percent of NSBRI costs are expended on research, 7 percent on education and public outreach, and 8 percent on headquarters administration.

3.0 SUMMARY OF FY 2003 ACTIVITIES

A summary of major NSBRI activities taking place in FY 2003 appears in Table 1.

Table 1. MAJOR NSBRI ACTIVITIES October 1, 2002 – September 30, 2003		
DATE	ACTIVITY	LOCATION
October 18	First Meeting of NASA/NSBRI Steering Group	Houston
November 16-22	NSBRI Education and Public Outreach Team Representatives attend OBPR Annual Education/Public Outreach Retreat	Missoula, MT
January 1	NSBRI Headquarters Chief of Staff Hired	N/A
January 13-15	NASA Bioastronautics Investigators Workshop	Galveston, TX
February	Associate Director Resigns/Search Begins	N/A
February 19	Selection Meeting for NSBRI's NRA 01-OBPR-07 Proposals	Houston
February 26-27	External Advisory Council Meeting	Houston
March 27	Board of Directors Meeting	Houston
April 15	Release of Joint NASA/NSBRI NRA 03-OBPR-04	N/A
April 15	Release of NSBRI Call for Candidates CFC-03-01	N/A
April 16	NASA/NSBRI Integrated Bone Team Meeting	Houston
April 17-18	NSBRI Team Representatives attend NASA's Enterprise Strategy Workshop	Houston
May 5	NASA/NSBRI Radiation Steering Group Site Visit	Upton, NY
May 8	NSBRI Education and Public Outreach Advisory Group Meeting	Houston
June 15	Deadline for Applications for Associate Director	N/A
June 27	Revised Cooperative Agreement Management Plan Approved	N/A
July 15	Deadline for Submissions to NRA 03-OBPR-04 and NSBRI CFC-03-01	N/A
July 17	NSBRI Revised Strategic Plan Submitted to NASA	N/A
August 25-26	Search Committee Interviews Associate Director Candidates	Houston
September	Peer Review of NRA 03-OBPR-04 Proposals Begins	Washington, DC
September 3-4	External Advisory Council Meeting	Houston
September 10	Space Medicine Liaison Appointed	N/A
September 18	Board of Directors Meeting	Houston

4.0 STRATEGIC DIRECTIONS

The NSBRI revised its strategic plan in FY 2003. After vetting it through the EAC, Board of Directors and JSC, the Revised Strategic Plan (Appendix A) was signed, approved and delivered to NASA. The Revised Strategic Plan is well aligned with NASA's Bioastronautics Strategy, which was also approved by NASA Headquarters' Codes U, M and AM in FY 2003. The NSBRI Revised Strategic Plan is posted on the Institute's Web site www.nsbri.org.

The Institute's Policy on Team Leadership, which was under revision in FY 2002, was also approved by NASA in FY 2003. NSBRI Team and Associate Team Leaders played an important role in assisting Code U (Office of Biological and Physical Research) with the development of its enterprise strategy.

FY 2003 witnessed the implementation of an NSBRI/NASA Steering Committee to coordinate, plan and discuss strategic and tactical issues involving senior management at NSBRI and JSC's Space and Life Sciences Directorate (SLSD). Coordination of NSBRI activities within Code UB was ensured by the Director's participation in weekly Bioastronautics telecons involving JSC and NASA Headquarters personnel in Bioastronautics.

NSBRI played a leadership role in the refinement activities involving the Bioastronautics Critical Path Roadmap (BCPR), in preparation for NASA having the BCPR reviewed by the Institute of Medicine and the National Research Council. NSBRI has membership in each of the discipline areas, as well as on the Bioastronautics Science Management Team and as part of Bioastronautics Management.

In June 2003, a revised CAMP (Appendix B) was approved to fully align the NSBRI/NASA management plan with the Institute's Revised Strategic Plan and NASA's Bioastronautics Strategy.

5.0 RESEARCH PROGRAM

5.1 Scientific and Technical Core Research Program and Achievements

Each research team consists of investigator groups working on complementary projects focused on a common theme. Team management is the responsibility of a program director called a Team Leader, who works with the Associate Team Leader to prepare and implement a team strategic plan. Team and Associate Team Leaders participate in monthly telecons with the Director and other NSBRI management to coordinate activities among the teams and with NASA. Additional focused meetings within and between teams occur throughout the year to refine the peer-reviewed research program and ensure that NSBRI countermeasure development research is clearly focused on high-risk areas and important critical questions on the BCPR.

In FY 2003, the NSBRI/NASA Steering Committee played an active role in bringing NSBRI teams and NASA investigators closer together and in integrating NSBRI research with NASA medical operations. Increased collaborative efforts among NSBRI and NASA investigators were evident at the Bioastronautics meeting held in January 2003. These efforts were also seen in the formation of new integrative research teams (e.g., bone loss) and in the formation of a space medicine team. These activities build upon the current 11 NSBRI research teams focusing on:

- *Bone Loss* – Addressing the loss and weakening of bone during space flight with the inherent fracture risks;

- *Cardiovascular Alterations* – Addressing inflight increase of cardiac dysrhythmias and postflight impairment of the cardiovascular response to orthostatic and exercise stress;
- *Human Performance Factors, Sleep and Chronobiology* – Investigating maintenance of high cognitive performance and vigilance despite environmental stress and sleep disturbances;
- *Immunology, Infection and Hematology* – Addressing immune system impairment and altered susceptibility to infection, increased allergic response, decreased blood volume and postflight anemia;
- *Muscle Alterations and Atrophy* – Focusing on the loss of skeletal muscle mass, strength and endurance that accompanies space flight;
- *Neurobehavioral and Psychosocial Factors* – Investigating methods and tools crews can utilize to cope with stress, isolation and compatibility;
- *Neurovestibular Adaptation* – Addressing the problems of space motion sickness and disorientation during flight and the postflight problems of balance and gaze disorders;
- *Nutrition, Physical Fitness and Rehabilitation* – Developing methods to maintain health and fitness before, during and after space flights;
- *Radiation Effects* – Addressing the problem of increased cancer risk caused by the natural space radiation environment;
- *Smart Medical Systems* – Developing new methods of remote medical monitoring, diagnosis and treatment; and
- *Technology Development* – Developing instrumentation that will enhance the research of the other teams and transferring the technology to industry for the benefit of society.

During FY 2003, three new ground projects were selected from NRA 01-OBPR-07. These projects augmented research on three NSBRI teams – Human Performance Factors, Sleep and Chronobiology; Muscle Alterations and Atrophy; and Nutrition, Physical Fitness and Rehabilitation. Drs. Richard Cohen and Jan Meck each had flight studies involving cardiovascular alterations and countermeasures funded, in coordination with JSC and NASA Headquarters. A complete listing of project executive summaries for the NSBRI core research program is contained in Appendix C. The research team reports for FY 2003 are in Appendix D.

Individual projects, in addition to the teams, submit annual reports on progress, which are reviewed by the BSC. Evaluations from the BSC, along with a summary of research activities and progress, are presented to the EAC. NASA representatives are invited to the semi-annual EAC meetings. The EAC chair and the NSBRI Director report to the Board of Directors semi-annually. The Director's reports for the March 2003 and September 2003 Board of Directors Meetings are included in Appendix E.

A listing of NSBRI publications and presentations during FY 2003 appears in Appendix F. In addition to the productivity and achievements contained therein, efforts were made in FY 2003 to strengthen the countermeasure development pipeline, as products mature towards evaluation, validation and operation. Members of the Technology Development, Smart Medical Systems and Bone Loss Teams, in conjunction with the Industry Forum and representatives of the SLSD, Engineering Directorate and Astronaut Office at JSC, met on several occasions to foster bilateral communications regarding operational needs, requirements and opportunities and NSBRI research and resources.

5.1.a. Research Announcements

Following the precedent established in FY 2001 with the release of NRA 01-OBPR-07, wherein NSBRI and NASA coordinated their research solicitations for proposals in the Biomedical Research and Countermeasures program, the Institute and NASA worked together to release a joint solicitation in FY 2003. The solicitation, NRA 03-OBPR-04, is included as Appendix G.

The NRA was widely disseminated, including prominent displays on the NSBRI and NASA Web sites.

NRA 03-OBPR-04 requested proposals to 10 NSBRI teams, which included all research teams except for the Radiation Effects Team. This team is undergoing evolution to better align it with other NASA radiation efforts, including investment in the NASA Space Radiation Laboratory at the U.S. Department of Energy's Brookhaven National Laboratory.

In preparing NRA 03-OBPR-04, the Institute clearly emphasized the need for team-based countermeasure research and development that was translational and at the mid-range countermeasure readiness levels. All proposals were required to link research to the BCPR and its critical questions.

The coordination between NSBRI and NASA extended to other NRAs released in FY 2003. For example, NSBRI language appeared in NRA 03-OBPR-06 concerning Ground-Based Analogs of Space Flight.

NSBRI's electronic submission system, used for proposals to the Institute's portion of NRA 03-OBPR-04, worked well. Both NASA and NSBRI submissions to NRA 03-OBPR-04 are undergoing peer review for merit using the same criteria of scientific excellence.

5.1.b. Team Leadership

The NSBRI released a Call for Candidates in FY 2003 soliciting applications for team leadership on the 10 teams identified in NRA 03-OBPR-04. The Call for Candidates was issued separately from the NRA. The NSBRI worked with NASA to ensure that team leadership positions would be re-competed and that a Policy on Team Leadership, approved by both NSBRI and NASA, was adopted and implemented. The Call for Candidates appears in Appendix H. The Policy on Team Leadership is part of the Call for Candidates and is also included as an appendix to the NSBRI Revised Strategic Plan.

5.2 Core Space Medicine Program Achievements

In FY 2003, NSBRI made significant progress expanding efforts in its core space medicine program. The Medical Operational Support Team project began to be used for training by NASA flight surgeons. Dr. Jonathan Clark, a NASA flight surgeon, was detailed part-time by the Space Medicine and Health Care Systems Office at JSC to serve as the NSBRI/NASA Space Medicine Liaison. A working list of flight surgeon assignments to each of the NSBRI research teams was made. The need for a Behavioral Medicine Liaison, to help link psychological research and care to the rest of space medicine across the NSBRI/NASA interface, was identified. Recruitment began for this position. The incumbent will also be member of the Human Performance Factors, Sleep and Chronobiology Team and the Neurobehavioral and Psychosocial Factors Team.

The Institute continued to sponsor its space medicine workshops and co-issued, with NASA, a report on Resuscitation and Critical Care in Space (Appendix I). Through Baylor College of Medicine, the NSBRI fostered the approval of continuing medical education credits for space medicine rounds. These rounds bring NSBRI researchers and the user community at JSC in direct contact with each other on a regular basis to discuss operationally valid countermeasures.

Advances were made in the scientific and medical use of the ultrasound device aboard the Human Research Facility on the International Space Station (ISS). Activities involving crews of ISS Increments 7 and 8 were conducted, with future joint NASA/NSBRI ultrasound activities planned for FY 2004 and beyond.

5.3 Partnerships: NASA, Other Institutions and International

To enhance the working partnership involving NSBRI and NASA biomedical research, a number of initiatives were taken in FY 2003. These include the implementation of the NSBRI/NASA Steering Group, the formation of a NSBRI/NASA Radiation Steering Group, bilateral participation in revising strategic and tactical documents, the development and release of joint research announcements, the formation of integrated research teams, and the creation of NSBRI/NASA liaison positions in space medicine and behavioral medicine.

The NSBRI continued to engage its consortium institutions and broadened its base of funded investigators to represent 81 academic institutions or universities. Some investigators have affiliations with foreign universities. NSBRI is continuing its efforts on the international artificial gravity project, which is being coordinated through NASA Headquarters.

6.0 EDUCATION AND PUBLIC OUTREACH PROGRAM

The NSBRI program in education and public outreach supports the Institute's mission by ensuring open involvement in the Institute's activities by the scientific community, industry and the public, and by adding unique value to NASA's educational programs, coordinated through Codes N and U.

Initially, NSBRI education and public outreach activities were led by teams at three consortium institutions: Morehouse School of Medicine, Texas A&M University and Baylor College of Medicine. An active Communications and Outreach group, run through NSBRI Headquarters, also has a vital role in disseminating information regarding the Institute's activities and achievements.

In response to an NSBRI Special Program Announcement issued in FY 2000, seven projects were selected for funding to form the current Education and Public Outreach Team. This team focuses on K-16 projects, including programs for teacher development and training, student educational opportunities, teacher and student materials in space life sciences, graduate student curriculum development and science-writing courses. Summaries of the team's projects are presented in Appendix J.

The Education and Public Outreach Team made significant progress in FY 2003 and a separate report of the team's accomplishments appears in Appendix K. The NSBRI hosted a series of teacher workshops involving more than 2,300 teachers from across the country. A summary of these workshops and of conference outreach is shown in Appendix L. Additionally, the Institute ran a successful and popular summer internship program in FY 2003, where 12 students were matched to NSBRI and NASA investigators who were mostly working at JSC. A list of summer interns and mentors, along with final project reports, is contained in Appendix M.

NSBRI education team members participated in a Code U annual retreat for education, and reciprocally, JSC and NASA Headquarters educators attended key NSBRI Education and Public Outreach programs. During the second half of FY 2003, the Institute undertook a series of planning activities regarding education and the need to strengthen the career development and training pipeline from kindergarten through independent investigator, as presented in the Revised Strategic Plan.

To help strengthen the pipeline, the Institute is implementing a postdoctoral program in FY 2004. The program proposal received favorable review from the BSC in FY 2003. Fellowships will be awarded on a competitive basis, and fellows will help link NSBRI and NASA biomedical research activities as part of the program's efforts to support the next generation of space life scientists.

7.0 MANAGEMENT

7.1 Organization and Key Personnel

As Institutions develop, the management structure often requires refinement to best meet the mission, as well as the expectations and investments of its stakeholders. The NSBRI is a complex organization. During FY 2003, several management changes were discussed and implemented.

As previously mentioned, an NSBRI/NASA Steering Group was implemented to provide a forum for senior NSBRI and SLSD management to meet on a regular basis. A Chief of Staff position was created and filled to bridge activities between the Office of the Director, the Chairman of the Board, Baylor College of Medicine and JSC. A Manager for Information Technology was appointed, given the Institute's growing reliance on electronic management of research, education and finance. A new position in metrics was identified and filled, with direct reporting to the Manager for Information Technology.

In addition to strengthening the administrative management team, the Institute expanded its management of science and medicine activities. While preserving the important research team structure with its Team and Associate Team Leader positions, liaison positions were added to bridge (a) NSBRI and NASA biomedical science, (b) space life sciences research and medical operations, and (c) space medicine and behavioral health. Dr. Jonathan Clark filled the NSBRI/NASA Space Medicine Liaison position. It is anticipated that the Behavioral Medicine Liaison position and the Science Integration Lead/Manager position will be filled in early FY 2004. A schematic of the revised NSBRI organizational structure is shown in Table 2.

Organizational changes are often accompanied by personnel changes, and in February 2003, Dr. Ronald White resigned as Associate Director. A combined NSBRI/NASA Search Committee was formed and several promising finalists for this leadership position are under consideration.

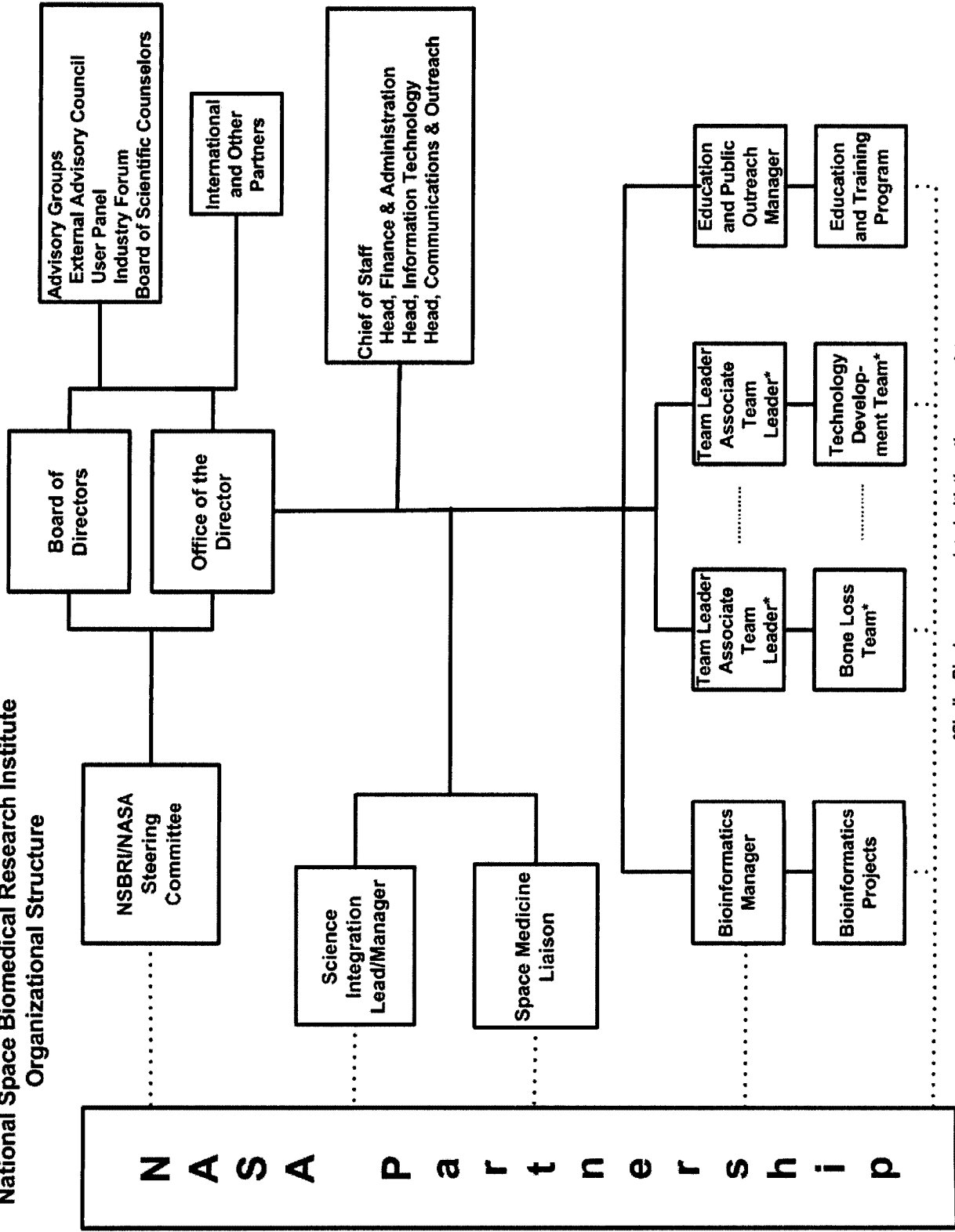
7.2 Board of Directors

The current membership of the NSBRI Board of Directors appears in Table 3. The Board convened two times in FY 2003. The meetings were held in Houston.

Three new members were added, either filling a vacancy or replacing a member who stepped down. New members included Dr. Craig J. Hogan, University of Washington; Hon. John E. Porter, Hogan & Hartson; and Dr. Frederick B. Rudolph, Rice University. Board members stepping down included Dr. Susanne E. Churchill, Harvard Medical School; Dr. Alvin L. Kwiram, University of Washington; and Dr. Larry McIntire, Rice University.

Table 2. NSBRI ORGANIZATIONAL STRUCTURE

**National Space Biomedical Research Institute
Organizational Structure**



*Similar Blocks are associated with the other research teams

Table 3.

NSBRI BOARD OF DIRECTORS

Bobby R. Alford, M.D. (Chairman) Baylor College of Medicine	William L. Allen National Geographic Magazine	Carl W. Anderson, Ph.D. Brookhaven National Laboratory
Thomas E. Andreoli, M.D. University of Arkansas College of Medicine	James B. Bassingthwaite, M.D., Ph.D. University of Washington	Joseph V. Bonventre, M.D., Ph.D. Harvard-MIT Division of Health Sciences and Technology Harvard Medical School
James F. Buchli United Space Alliance	Michael E. DeBakey, M.D. Baylor College of Medicine	Richard E. Ewing, Ph.D. Texas A&M University
Alfred P. Fishman, M.D. University of Pennsylvania Health System	Theresa W. Fossum, D.V.M., Ph.D. Texas A&M University	Alice P. Gast, Ph.D. Massachusetts Institute of Technology
Glen N. Gaulton, Ph.D. University of Pennsylvania School of Medicine	Martha L. Gray, Ph.D. MIT-Harvard Division of Health Sciences and Technology Massachusetts Institute of Technology	E. Nigel Harris, M.D. Morehouse School of Medicine
Craig J. Hogan, Ph.D. University of Washington	Richard J. Johns, M.D. The Johns Hopkins University School of Medicine	Joseph P. Kerwin, M.D. Wyle Laboratories
Steven Knapp, Ph.D. The Johns Hopkins University	Jordan Konisky, Ph.D. Rice University	J. David Litster, Ph.D. (Emeritus) Massachusetts Institute of Technology
Lawrence A. Palinkas, Ph.D. (<i>Ex Officio</i>) University of California, San Diego	James W. Patrick, Ph.D. Baylor College of Medicine	Peter Paul, Ph.D. Brookhaven National Laboratory
Hon. John E. Porter Hogan & Hartson	Mary R. Rifkin, Ph.D. Mount Sinai School of Medicine	Frederick B. Rudolph, Ph.D. Rice University
Alan L. Schiller, M.D. Mount Sinai School of Medicine	Kenneth I. Shine, M.D. Rand Corporation	Walter W. Sullivan, Ph.D. Morehouse School of Medicine
Jeffrey P. Sutton, M.D., Ph.D. (<i>Ex Officio</i>) Institute Director	W. Dalton Tomlin (Secretary/Treasurer) Baylor College of Medicine	Arnold N. Weinberg, M.D. (Emeritus) Massachusetts Institute of Technology
Torsten N. Wiesel, M.D. (Emeritus) Rockefeller University	I. Dodd Wilson, M.D. University of Arkansas for Medical Sciences	

7.3 External Advisory Council

The current membership of the NSBRI External Advisory Council appears in Table 4. The Council met two times in Houston in FY 2003. Dr. Martin J. Fettman, Colorado State University, stepped down as chair of the EAC. Dr. Lawrence A. Palinkas, University of California, San Diego, was appointed to replace him.

Ten new members were added, either filling a vacancy or replacing a member who stepped down. New members included Dr. Gilbert A. Castro, The University of Texas Health Science Center at Houston; Dr. David Costill, Ball State University; Dr. Stephen Doty, Hospital for Special Surgery; Dr. Gregory T. A. Kovacs, Stanford University School of Medicine; Dr. R. Bruce Martin, University of California, Davis; Dr. Donna M. Murasko, Drexel University; Dr. Carolyn F. Randolph, South Carolina Governor's School for Science and Mathematics; Dr. Muriel D. Ross, The University of New Mexico Health Sciences Center; Dr. William Schwartz, University of Massachusetts Medical School; and Dr. Charles M. Tipton, University of Arizona College of Medicine.

Four members rotated off the Council having served five years – Dr. Michael N. Gould, University of Wisconsin Medical School; Dr. Danny A. Riley, Medical College of Wisconsin; Dr. Thomas J. Wronski, University of Florida; and Dr. Bill J. Yates, University of Pittsburgh. Three members stepped down due to other commitments – Dr. J. A. Anderson, Brown University; Dr. Victor A. Convertino, U.S. Army Institute of Surgical Research; and Dr. Charles B. Nemeroff, Emory University.

7.4 Board of Scientific Counselors

During FY 2003, the Board of Scientific Counselors was revitalized to include members in all discipline areas. BSC members serve on combined NASA/NSBRI peer review panels.

7.5 User Panel

The User Panel lost a member during FY03 when Dr. Laurel S. Clark died in the STS-107 accident.

8.0 SUPPORTING PROGRAMS

8.1 Industry Forum

The NSBRI commissioned a report in FY 2003 from the Massachusetts Institute of Technology regarding commercializing NSBRI-specific intellectual property. The report, which appears in Appendix N, examines three options. The Industry Forum agreed that a combination of two options – increased awareness and targeted communications – is an appropriate path for the Institute to follow. Steps are being taken to implement this action.

During FY 2003, the Industry Forum membership was expanded to include Novartis Pharmaceuticals Corporation (Table 5).

Table 4.

NSBRI EXTERNAL ADVISORY COUNCIL

<p>Lawrence A. Palinkas, Ph.D. (Chairman) Professor Family and Preventive Medicine University of California, San Diego</p>	<p>Leon Alkalai, Ph.D. Director Center for Integrated Space Microsystems Jet Propulsion Laboratory</p>	<p>Ruth Benca, M.D., Ph.D. Professor and Associate Chair Department of Psychiatry University of Wisconsin Medical School</p>
<p>Theodore W. Berger, Ph.D. Professor Biomedical Engineering University of Southern California</p>	<p>Hal. E. Broxmeyer, Ph.D. (<i>Ex Officio</i>) Chairman and Professor Microbiology/Immunology Indiana University School of Medicine</p>	<p>Thomas F. Budinger, M.D., Ph.D. Professor and Chair Department of Bioengineering Lawrence Berkeley National Laboratory</p>
<p>Gilbert A. Castro, Ph.D. Interim Executive Vice President Academic Affairs University of Texas Health Science Center at Houston</p>	<p>Dennis S. Charney, M.D. Chief of Mood and Anxiety Disorders Research Program National Institute of Mental Health</p>	<p>David Costill, Ph.D. John and Janice Fischer Chair in Exercise Science Ball State University</p>
<p>Stephen Doty, Ph.D. Senior Scientist Hospital for Special Surgery</p>	<p>Thomas A. Fleisher, M.D. Chief, Department of Laboratory Medicine National Institutes of Health</p>	<p>Eileen M. Hasser, Ph.D. Professor, Veterinary Biomedical Sciences University of Missouri-Columbia</p>
<p>Gregory T. A. Kovacs, M.D., Ph.D. Associate Professor, Electrical Engineering Stanford University School of Medicine</p>	<p>Amy Kronenberg, Sc.D. Group Leader Radiation Biology and Environmental Toxicology Lawrence Berkeley National Laboratory</p>	<p>R. Bruce Martin, Ph.D. Professor and Director Orthopaedic Research Laboratories University of California, Davis</p>
<p>Donna M. Murasko, Ph.D. Professor and Interim Dean, College of Arts and Sciences Bioscience and Biotechnology Drexel University</p>	<p>Carolyn F. Randolph, Ph.D. Vice President for Outreach and Research South Carolina Governor's School for Science and Mathematics</p>	<p>Irwin H. Rosenberg, M.D. Professor of Medicine and Nutrition Tufts University</p>
<p>Muriel D. Ross, Ph.D. Adjunct Professor Department of Neuroscience The University of New Mexico Health Sciences Center</p>	<p>William Schwartz, M.D. Professor of Neurology, Graduate School of Biomedical Sciences University of Massachusetts Medical School</p>	<p>M. Rhea Seddon, M.D. Assistant Chief Medical Officer Vanderbilt University Medical Center</p>
<p>Jeffrey P. Sutton, M.D., Ph.D. (<i>Ex Officio</i>) Institute Director</p>	<p>Charles M. Tipton, Ph.D. Professor Emeritus Department of Physiology and Surgery University of Arizona College of Medicine</p>	<p>F. Eugene Yates, M.D. Professor of Medicine Medical Monitoring Unit University of California, Los Angeles</p>

Table 5.

NSBRI INDUSTRY FORUM MEMBERSHIP

<p>The Boeing Company The Charles Stark Draper Laboratory InDyne, Inc. Lockheed Martin Astronautics MBI International Novartis Pharmaceuticals Corporation Payload Systems, Inc. Raytheon Technical Services Company Roche Laboratories, Inc. SGI Southwestern Bell United Space Alliance Veridian Wyle Laboratories</p>
--

8.2 Bioinformatics

During FY 2003, the NSBRI expanded its efforts in metrics to map activities and monitor findings, with respect to the BCPR and critical questions. Reporting systems were developed and implemented for both the core and non-core (translational workforce) research programs. These systems augment information being acquired through the NSBRI Data Archive system, which was established in FY 1998. As the information technology infrastructure of the Institute expands and NSBRI and NASA efforts in bioinformatics become more integrated, the NSBRI will be able to achieve its goals and objectives in bioinformatics as stated in the Revised Strategic Plan.

8.3 Communications and Outreach

In support of NSBRI's mission, the Communications and Outreach Office develops and implements diverse communications and outreach initiatives. The program identifies and targets messages to the NSBRI's key publics – the scientific community, industry, consortium members, the general public and NASA. Key activities in FY 2003 included the continuation of a research-based news release program, the collaboration with public affairs offices at numerous funded institutions to maximize news outreach related to the NSBRI research program, meetings with JSC public affairs to increase awareness of NSBRI activities, exhibits at two key space symposiums, and an increase in news placements related to NSBRI's space-related research.

In FY 2003, the Institute received 107 media inquiries seeking interviews with NSBRI investigators. Similarly, 225 newspaper, magazine or on-line articles mentioned NSBRI, up from 204 the previous year. Since the NSBRI does not utilize a news clipping service, the clipping

figure is based on material received from reporters and other sources. Subscriptions to the NSBRI's Web-based E-News Service increased to 653, up from 386 at the end of FY 2002.

Key NSBRI research information appeared in the New York Times, Scientific American, Popular Science, Discover Magazine, Dallas Morning News, Nature, CNN.com, Space.com, Florida Today, Houston Business Journal, Houston Chronicle, Fort Worth Star-Telegram and Seattle Times. In FY 2003, the Communications and Outreach program targeted trade publications in an attempt to engage a science literate segment of the population that might not be well informed about space research. NSBRI research feature articles in this sampling of science/medical trade publications: RSNA News (Radiological Society of North America), Scientific Computing World, Orthopedic Technology Review, Access Audiology, Mechanical Engineering Magazine and Spectroscopy Now.

9.0 INSTITUTE DIVERSITY AND SCIENTIFIC COMMUNITY OUTREACH

The NSBRI continues to build diversity and engage a broad scientific community through outreach efforts. NRA 03-OBPR-04 (Appendix G) and the Call for Candidates (Appendix H) are open solicitations and information regarding them was widely disseminated. There was diversity in the FY 2003 summer internship program (Appendix M), and the Institute has paid careful attention to diversity in revitalizing its BSC, which must also meet with NASA's approval since BSC members serve on joint NSBRI/NASA review panels.

Morehouse School of Medicine continued to have a leadership role in NSBRI education programs in FY 2003, and the postdoctoral fellowship program will be open and encourage diversity.

NSBRI investigators, including Team and Associate Team Leaders, and management continued to reach out to the scientific community through presentations made at symposia and meetings. The Institute had wide visibility at the World Space Congress, Humans in Space Symposium, American Society for Photobiology, International Gravitational Physiology Meeting, and the Joint Meeting of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, as well as other prominent professional meetings.

10.0 SPECIAL PROJECTS

The CAMP between NASA and the NSBRI enables the partners to undertake special projects outside the core-funding envelope of the NSBRI. During FY 2003, two new projects began and 14 projects continued. Detailed information on the two new projects and three projects that experienced change is provided.

Project 97-3, *National Space Biomedical Research Institute Visiting Scientist/Research Associate Program*, continues to enable young and established university-based researchers an opportunity to work side-by-side with government employees in JSC laboratories. Table 6 provides a list of the participants in this program and other projects relating to Bioastronautics positions at JSC.

Table 6. VISITING SCIENTIST/RESEARCH ASSOCIATE PROGRAM – FY 2003

Name	NASA Program Area	Period
Jennifer Blume, Ph.D. (see Project 00-1)	Habitability and Environmental Factors Office	1/4/00 - 5/19/03
Tatiana Christian	Habitability and Environmental Factors Office	5/22/00 -
Johnny Conkin, Ph.D.	Environmental Physiology Laboratory	6/1/98 -
Richard Danielson, Ph.D. (see Project 03-3)	Operational Hearing Conservation Project	1/13/03 -
Dominick D'Aunno, M.D.	Cardiovascular Physiology Laboratory	11/1/97 -
John N. Evanoff, Ph.D.	Human Adaptation and Countermeasures Office	7/23/01 - 1/11/03
Philip Foster, M.D., Ph.D.	Environmental Physiology Laboratory	10/19/98 - 9/17/03
Wendy Garner, Ph.D.	Cardiovascular Physiology Laboratory	11/24/97 - 5/27/03
Todd Hellner	Habitability and Environmental Factors Office	9/24/01 -
Meena Husein	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	6/1/01 -
Ralph Krog, J.D.	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	9/17/01 -
Lawrence H. Kuznetz, Ph.D.	Human Adaptation and Countermeasures Office	8/20/01 -
Ajitkumar Mulavara, Ph.D.	Neuroscience Laboratory	8/20/01 -
John B. Peacock, Ph.D.	Habitability and Environmental Factors Office	10/30/00 -
Michele Perchonok, Ph.D.	Habitability and Environmental Factors Office	9/5/00 -
Lanny Rudner	Human Adaptation and Countermeasures Office	6/17/02 - 4/16/03
Sudhakar Rajulu, Ph.D.	Habitability and Environmental Factors Office	4/17/00 -
Lawrence Spector	Habitability and Environmental Factors Office	9/25/00 -
M. G. Sriram, Ph.D. (see Project 03-2)	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	12/9/02 -
Hong Lu Wu, Ph.D. (see Project 02-5)	Space Radiation Health Project Office	4/1/03 -
Cary J. Zeitlin, Ph.D.	Space Radiation Health Project Office	5/13/02 -

Project 00-1, *Human Factors Engineering & Operational Habitability*, is a project designed to enable the NSBRI and JSC to develop joint activities related to space habitability and applied human factors research. Development of a more thorough understanding of these research areas, which are not part of the Institute's core research activity, will enhance the Institute's ability to develop future research programs that will provide the critical data necessary to promote human engineering and habitability for space flight. Dr. Jennifer Blume left this position in May 2003. The project position is vacant at this time.

Project 02-5, *Experimental Radiation Biologist*, provides an experimental radiation biologist to work with the JSC Radiation Biophysics Laboratory and the Space Radiation Health Project (SRHP) to provide leadership in developing new methods in cytogenetics, biomarkers and the studies of genomic instability. Duties include developing protocols for understanding the origin and outcome of clonal aberrations found in blood samples, supporting the biodosimetry activities ongoing in the Radiation Biophysics Laboratory and coordinating the research integration efforts

of the SRHP. Recruiting efforts for this position began at the end of FY 2002. NSBRI, in cooperation with JSC management, hired Dr. Hong L. Wu in FY 2003.

Project 03-2, *Medical Informatics and Healthcare Systems Evidence-Based Medicine and Medical Informatics Application Development Project*, provides an academically qualified and experienced software systems architect, knowledge engineer and statistician to the Medical Informatics and Healthcare Systems (MIHCS) Group at JSC. This position is responsible for leading application/system architecture research, design and development in Web-based clinical databases for data mining and knowledge discovery in support of evidence-based medicine and medical informatics. In addition, he develops and manages computer projects using knowledge-based techniques, mathematical/statistical modeling, biomedical knowledge representation, databases, application programming, Web applications and algorithmic methods. To carry out this project, the NSBRI, in cooperation with JSC MIHCS management, hired Dr. M. G. Sriram for the position of Senior Software Systems Architect.

Project 03-3, *NASA Operational Hearing Conservation Project*, provides an internationally recognized expert in the field of audiology and hearing conservation to JSC to provide oversight and expertise to every facet of hearing conservation and preventive/diagnostic audiology. This person provides leadership and human communications expertise that is relevant to the Critical Path Roadmap for solving current communications and acoustics issues and prepares the agency for future mission-related auditory and communications challenges. He is responsible for bringing together all parties at JSC who have some stake in hearing conservation, acoustics and human communications issues, including scientists, physicians, allied health professionals, acoustic engineers, mechanical engineers, the astronaut corps, etc. NSBRI, in cooperation with NASA Bioastronautics Program management, hired Dr. Richard Danielson.

11.0 FUTURE DIRECTIONS

The NSBRI Revised Strategic Plan (Appendix A) delineates future directions in countermeasures development research and in education, training and public outreach. These directions are consistent with the CAMP (Appendix B), the Bioastronautics Strategy and the Biological and Physical Research Enterprise (Code U) Strategy.

The Institute will build on its research strengths embodied in the team structure as described in the Revised Strategic Plan. Research efforts in Technology Development, Radiation Effects, Space Medicine and Modeling/Bioinformatics will become cross-cutting, rather than discipline, teams. Programmatic continuity of meritorious and relevant projects is a high priority for the Institute. Strategic and tactical considerations have been discussed and vetted throughout FY 2003, in order to facilitate a smooth transition in the research program in FY 2004.

Going forward, there will further refinement of the BCPR and a tighter link of the research program to this roadmap. Progress concerning countermeasure development and deliverables that help answer critical questions will be tracked more thoroughly. Future NRAs with NASA will focus on gaps in the research program and will provide opportunities for program project-type grants which address specific critical questions. There will also be a process for peer-reviewed synergy grants, which can be awarded through efficient use of the Institute's BSC and EAC prior to selection.

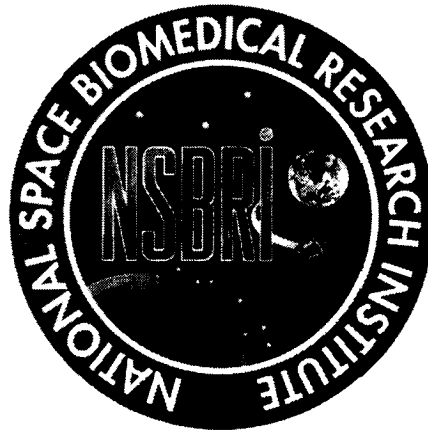
Research integration with NASA will increase, with emphasis on higher countermeasure readiness levels and transition towards evaluation, validation and flight. Bidirectional linkages between the NSBRI research community and space medicine will also become stronger, especially with the NSBRI/NASA Space Medicine and Behavioral Health Liaisons in place to facilitate translational tasks faced by the various discipline teams. There also will be increased integration between the core and non-core components of the NSBRI research program.

The postdoctoral training program will become operational in FY 2004. A solicitation for K-16 educational projects and graduate curriculum development will be developed to enable the NSBRI to continue a strong program in education and public outreach.

Finally, the Institute's management reorganization will be completed in FY 2004. This will give the Institute broader scientific and administrative support, while preserving the importance of the team leaders, to increase productivity, build on opportunities and successfully meet challenges presented in the year ahead.

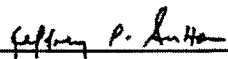
Appendix A

National Space Biomedical Research Institute



**REVISED
STRATEGIC PLAN**


July 17, 2003



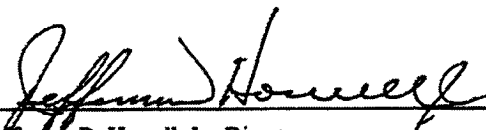
Jeffrey P. Sutton, M.D., Ph.D. 7/17/03
Director, National Space Biomedical Research Institute Date



Bobby R. Alford, M.D. 7/17/03
Chairman of the Board and CEO, National Space Biomedical Research Institute Date



Jeffrey R. Davis, M.D. 7/22/03
Director, Space and Life Sciences Directorate Date
NASA Johnson Space Center



Jefferson D. Howell, Jr., Director 8/15/03
NASA Johnson Space Center Date

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Introduction

The National Space Biomedical Research Institute (NSBRI) Strategic Plan is bold, forward-looking, and science-driven. It contains integrated and interconnected programs and education programs in Countermeasures Development Research and Education, Training, and Public Outreach. In partnership with NASA, the Institute's programs and goals provide unprecedented capabilities for focused, outcomes-driven research that leads to the development of effective countermeasures to mitigate the adverse effects of the space environment on crews and to ensure safe, long-duration human space flight.

The NSBRI/NASA partnership unites the expertise and resources of the broad biomedical research community with NASA's engineering and operational capabilities. The programs emphasize the importance of integrated research teams and encourage productivity and diversity at all levels. The Institute's mission is consistent with NASA's Bioastronautics Strategy, namely managing risk, increasing efficiency, and returning benefits to Earth. The Institute supports the Agency's mission-driven and enabling goals, especially, "to extend the duration and boundaries of human space flight to create new opportunities for exploration and discovery" and assists NASA in fulfilling its overall vision "to improve life here, to extend life to there, to find life beyond."

Mission

The mission of the National Space Biomedical Research Institute is to lead a national effort for accomplishing the integrated, critical path, biomedical research necessary to support long-term human presence, development, and exploration of space and to enhance life on Earth by applying the resultant advances in human knowledge and technology acquired through living and working in space.

Vision

The vision of NSBRI is to conduct world-class space biomedical research using innovative science, technology, and management strategies. The Institute, in partnership with NASA, will develop effective countermeasures that substantially reduce significant biomedical risks associated with human space travel. These discoveries will ensure crew health and well-being by mitigating the adverse effects of the space and microgravity environment. They will also improve life on Earth. The Institute will engage a diverse, open community of outstanding scientists, engineers, and clinicians to work on peer-reviewed projects in integrated research teams using the resources of leading institutions to achieve the Institute's mission and inspire the next generation of space life scientists. The Institute will be the focal point of, and major resource for, NASA-sponsored space biomedical research.

Operational Concept and History

NSBRI is a unique, non-profit scientific partnership established in 1997 following competitive selection by NASA. Since its inception, and in collaboration with the Johnson Space Center (JSC) through a Cooperative Agreement, NSBRI has initiated and cultivated its leadership role in space biomedical research through novel strategies to meet its mission, goals, and objectives as set forth by NASA.

The Institute engages, facilitates, and coordinates outstanding academic, government, and industry researchers and educators in a team-based effort to develop countermeasures to reduce and/or eliminate health risks associated with human space travel. It also leverages the resources of the nation's leading biomedical research institutions and industry, allowing the combined intellectual and infrastructural capabilities to advance NASA's biomedical research program in an unprecedented manner. NSBRI is well positioned to efficiently and effectively conduct ground and critical in-flight studies of high relevance and impact for NASA, with tangible benefits for people on Earth. The Institute's operational concept is represented in Figure 1.

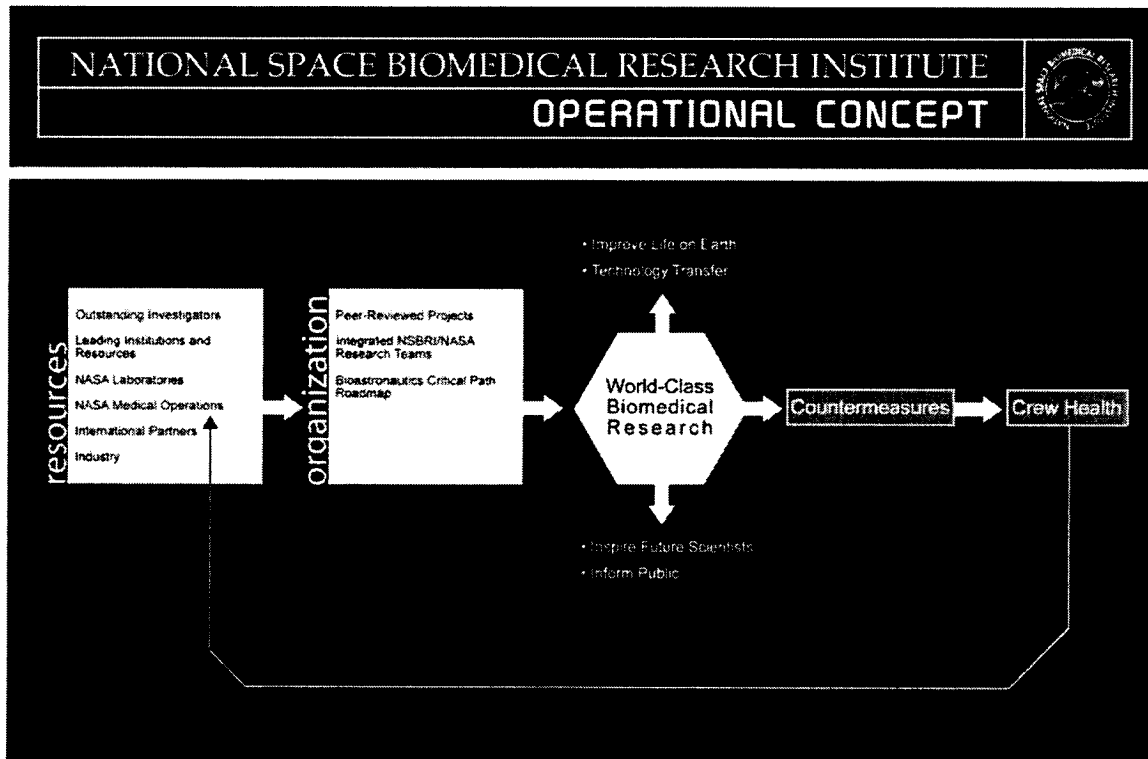
NSBRI was selected by NASA following a two-phase, competitive review of proposals received in response to the NASA Cooperative Agreement Notice 9-CAN-96-01. Seven academic institutions (Baylor College of Medicine, Harvard Medical School, The Johns Hopkins University School of Medicine and Applied Physics Laboratory, Massachusetts Institute of Technology, Morehouse School of Medicine, Rice University, and Texas A&M University) made up the initial Consortium governing the NSBRI. Baylor College of Medicine is the lead institution and houses the NSBRI headquarters.

In 1999, NASA approved an NSBRI augmentation plan. This plan was later incorporated in the Bioastronautics initiative established in the 2001 Presidential budget. The augmentation plan enabled the Institute to:

- Strengthen the original integrated research teams by increasing the number of tasks/teams.
- Identify and initiate new discipline research teams, including an increased focus on crew health.
- Open participation to the entire academic community.
- Accelerate countermeasure research in order to facilitate space flight studies.
- Increase emphasis on the Bioastronautics Critical Path Roadmap.
- Expand the education and public outreach program, consistent with Institute growth.
- Develop graduate and postdoctoral training programs.
- Increase emphasis on space biomedical data collection, evaluation, and consolidation.
- Grow Consortium membership through open competition.

The Consortium membership was expanded in 2000 from seven to 12 institutions following a competitive review process. Brookhaven National Laboratory, Mount Sinai School of Medicine, University of Arkansas for Medical Sciences, University of Pennsylvania Health System, and University of Washington were added to the Consortium.

**Figure 1
National Space Biomedical Research Institute
Operational Concept**



In November 2000, NASA’s Chief Scientist appointed a site visit committee to conduct a comprehensive review of NSBRI’s activities and progress in accordance with the Cooperative Agreement. The site visit Review Committee stated in their report:

“The committee was impressed by the scientific strengths of the NSBRI, by its progress in developing countermeasures, by its innovative scientific leadership and by its outstanding student trainees. We recommend that NSBRI continue for its second five-year funding period. We commend NSBRI for providing added value both for the Institute as a whole and for individual teams.”

The Institute’s Strategic Plan requested by the site visit Review Committee was submitted to NASA and reviewed in 2002. The Strategic Plan Review Committee noted:

“In the first five years of its existence, the Institute has made outstanding progress. It has:

- Developed a comprehensive research strategy that addresses key medical, physiological and technical issues associated with space travel;
- Recruited distinguished investigators from across the nation; and
- Established links to the key NASA end users, including astronauts, flight surgeons, engineers, other federal agencies, industry, and international partners.”

The Committee also noted:

“The benefits of the NSBRI Program to NASA and to health care on earth are potentially very significant. NASA must therefore commit to adequate and stable funding for the Institute to be able to develop effective countermeasures to enhance safety of prolonged and recurrent space travel.”

NASA/NSBRI Critical Issues and Strategies in Bioastronautics

The Institute is an integral constituent of NASA’s Bioastronautics effort, which is sponsored by the Office of Space Flight, the Office of Biological and Physical Research, and the Office of the Chief Health and Medical Officer. The Bioastronautics mission statement, as stated in the Bioastronautics Strategy, defines the human as a critical subsystem of space flight. The strategy endorses a risk-based approach that “allows for understanding and controlling the human health and performance risks, identifies specific outcomes, enables informed decision-making, and provides the Agency with highly developed risk management solutions to meet its enabling and mission-driven goals and objectives.” (Bioastronautics Strategy, p. 4) NSBRI significantly contributes to the implementation of the 11 Bioastronautics strategies.

Aims and Objectives

The Institute’s aims and objectives are to:

- Integrate the knowledge base relevant to the biomedical response of humans in space, understand and quantify the risk levels associated with this knowledge base, and recommend acceptable risk levels for long-duration missions. Risk levels in this context relate to present and future medical risk to the human participants as a result of deleterious effects of space flight, as well as to the subsequent risk to overall mission success.
- Develop and manage the implementation of an integrated research plan that will develop the required knowledge and technologies (across all biomedical and associated technological disciplines) to enable long-duration human space flight, including specific countermeasures where required.
- Implement a “best value” research program for the available resources.

- Demonstrate an understanding of the space medicine environment through an integrated on-site presence at JSC; feed back this knowledge to the discipline research teams.
- Facilitate science community access to the NASA space infrastructure associated with biomedical research.
- Develop and provide a science management process that will support the overall human in space biomedical research program.
- Ensure the dissemination of advances in knowledge resulting from this program to the scientific community.
- Promote and provide active collaboration with for-profit entities to ensure that developed technologies are transferred to the private sector.
- Conduct education and public outreach programs consistent with NSBRI's Mission and in support of NASA's education and public outreach objectives.

Programs and Goals

To fulfill its mission, vision, aims, and objectives, NSBRI has integrated and interconnected research and education programs that add unique value and complement NASA's Bioastronautics Strategy. These programs are in **Countermeasures Development Research, and Education, Training, and Public Outreach.**

Countermeasures Development Research Program

The goals of the program are:

- Lead a national peer-reviewed, integrated research effort to develop and test countermeasures that reduce biomedical risks of long-duration human space flight, and enable safe and productive missions and exploration of space.
- Provide directed medical and research capabilities that:
 - Support the transition to countermeasure validation and implementation, and the development of the Clinical Status Evaluation.
 - Create mechanisms for integrating and sharing knowledge and technologies.
 - Support an NSBRI/NASA translational workforce.
 - Establish strategic relationships and partnerships.
- Improve healthcare on earth and translate discoveries for possible commercialization.

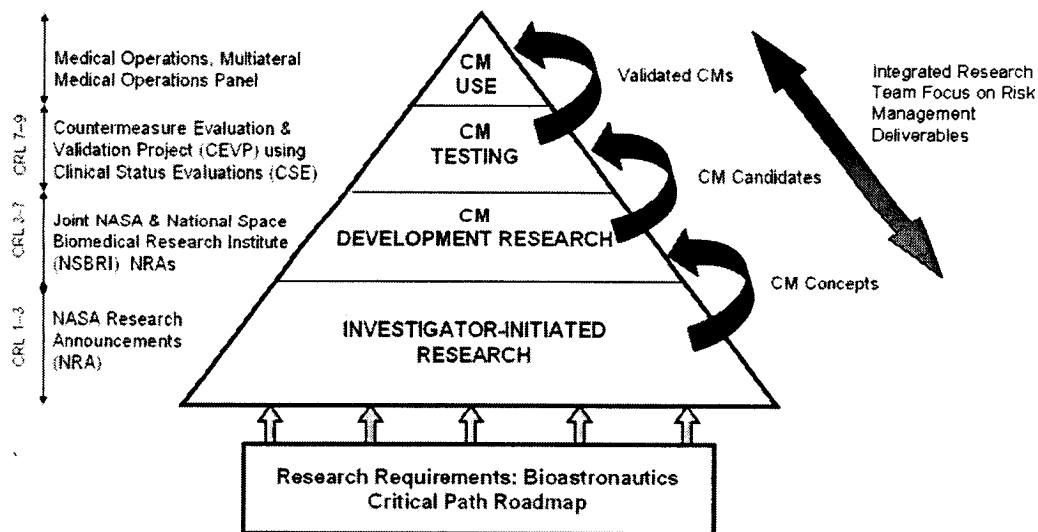
NSBRI research teams are developed and modified to provide a continuum of countermeasure research in the countermeasure readiness level (CRL 3-7 range that eventually leads to valid operational countermeasures that ensure crew health, safety, and performance. The teams complement and integrate with other NASA components of the Bioastronautics Program. To optimize the effectiveness, the Institute plans to develop cross-cutting efforts in radiation efforts, space medicine, technology and modeling. In addition, going forward, NSBRI research will advance to higher CRLs .

NSBRI Role in Bioastronautics

The development of countermeasures by NSBRI/NASA emanates from ideas and concepts that emerge from basic research and are then developed into research protocols and operational solutions (Figure 2). The process begins with the identification of the biomedical issues, risks, and priorities established through the CPR and progresses through a sequence of Countermeasure Readiness Levels (CRL). This includes basic and applied research to test and validate hypotheses (CRL 1-3), formulation of countermeasure concepts and initial demonstrations of efficacy (CRL 4-5), clinical trials and testing (CRL 6-7), and finally, validation and operational implementation (CRL 8-9). The CPR consists of 55 risks and the critical research questions/issues associated with each of the risks.

NSBRI countermeasures research focuses on CRL 3-7. To optimize its impact for NASA and generate outstanding deliverables for Bioastronautics risk management, NSBRI countermeasure research is coordinated and integrated with efforts in NASA medical operations and other components of the Bioastronautics program. The continuum from hypothesis-driven research, through the NSBRI and other NASA programs, to medical operations, forms the basis of an outcome and metrics-based model for risk assessment, risk reduction, and health care delivery. The Institute plays an important role in enabling and strengthening the Bioastronautics program to meet its risk management objectives.

Figure 2. Bioastronautics Countermeasure Development Process*



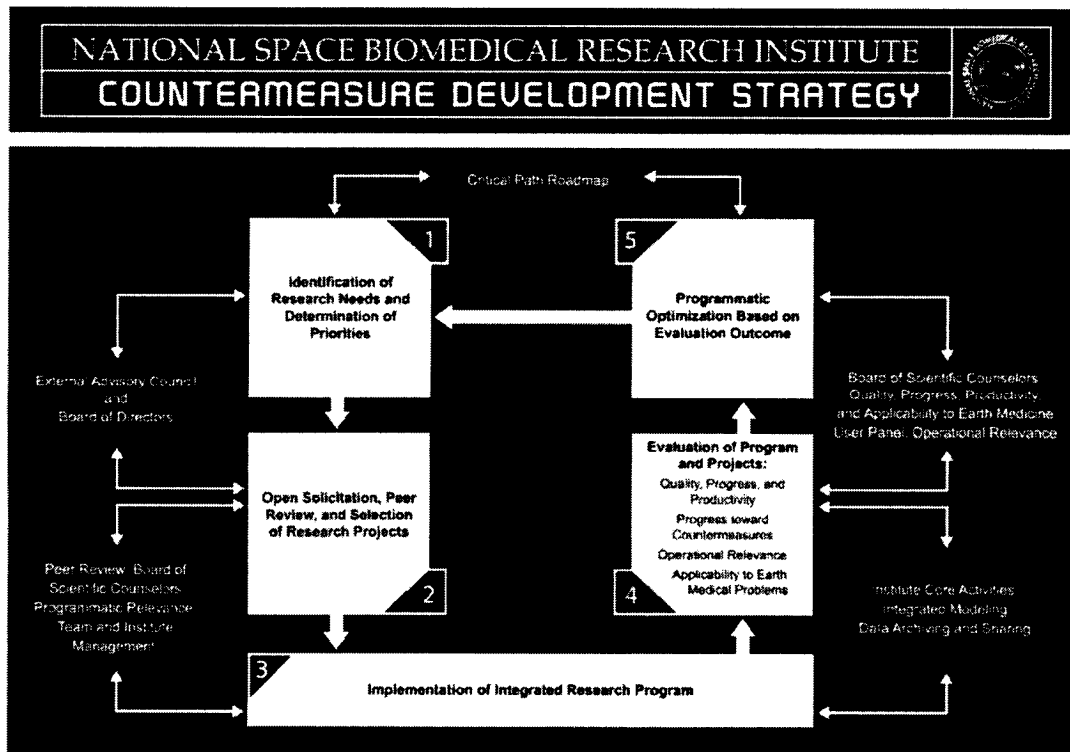
*Chart from the Bioastronautics Strategy, p. 5

NSBRI countermeasure development strategy involves five distinct, but related, steps in developing and managing its focused, integrated research program. These steps are based on the CPR and culminate in operational countermeasures. The strategies are:

- Identification of research needs and determination of priorities.
- Open solicitation, peer-review, and selection of research projects.
- Integrated research program.
- Evaluation of program and projects.
- Optimization of program based on evaluation outcome.

The Institute carries out these steps, as depicted in Figure 3, using an integrated, team-based research infrastructure. The approach has demonstrated effectiveness by enabling higher and higher levels of countermeasure readiness to be achieved in each of the high-priority research areas and by drawing outstanding new investigators to space-related problems. The strategy is coordinated with NASA at each step at multiple levels of NSBRI/NASA research and management. The strategic efforts in risk reduction are the responsibility of the NSBRI/NASA Steering Committee, comprised of senior NSBRI and JSC Space and Life Sciences personnel.

Figure 3.
Countermeasure Development Strategy



Research Priorities and Teams

The identification of research needs and determination of priorities are based on the CPR. NSBRI works interactively with JSC to ensure that its integrated research teams effectively complement NASA's research and operational efforts in CPR risk mitigation.

Currently, NSBRI's Countermeasures Development Research Program consists of eleven integrated discipline research teams: **Bone Loss; Cardiovascular Alterations; Human Performance Factors, Sleep and Chronobiology; Immunology, Infection and Hematology; Muscle Alterations and Atrophy; Neurobehavioral and Psychosocial Factors; Neurovestibular Adaptation; Nutrition, Physical Fitness and Rehabilitation; Radiation Effects; Smart Medical Systems; and Technology Development.** Each team has a strategic plan that summarizes the steps a team takes to develop specific countermeasures and technologies to manage biomedical risks. (See Table 1 of Appendix IV.) The plans

- Map specific risks on the CPR that a team addresses.
- List the risk-based and non-risk-based goals.
- Identify strengths, weaknesses, and gaps in the current team's program; and, set forth objectives and strategic activities for each goal.
- Provide the schedule and milestones for task completion given available resources..
- Describe the ways teams interact with other NSBRI teams and NASA to move concepts and deliverables through the countermeasure development process (Figure 2).

Two of these teams, radiation effects and technology development as well as space medicine, bioinformatics and modeling are cross cutting teams. These groups are a part of each of the integrated teams bridging each area to address the issues of the entire human system.

Project Selection

The Institute solicits ground-based research projects from the entire biomedical research community annually through open solicitations that are coordinated with, and approved by, NASA. The solicitations emphasize research areas that address gaps and issues identified in the **Team Strategic Plans**, and reflect high-priority critical questions in the CPR. Independent, peer-review panels of scientists with relevant expertise evaluate the proposals. These panels are selected by the same outside entity that NASA uses for its NASA Research Announcement (NRA) program. NSBRI selects an independent **Board of Scientific Counselors** (BSC) who are assigned to these panels according to their expertise. BSC members serve for several years in order to provide a corporate memory to the review process.

During peer review, proposals are scored for merit and evaluated for relevancy to the appropriate Team Strategic Plan and research solicitation topics. Proposals scoring within the competitive range are presented to the Institute's **External Advisory Council** (EAC) for discussion. Specific recommendations concerning the proposals, and their added value to the research teams and the entire program are made to NSBRI senior management (**Director and Associate Director**). Senior management is responsible for

selection, which is done in coordination with JSC and NASA Headquarters to maximize the overall benefit of the Institute to NASA's Bioastronautics program.

Flight NRAs for Bioastronautics are coordinated with JSC, NASA Headquarters and NSBRI and are selected in a standard process involving multiple steps, including definition and feasibility assessments.

Integrated Research Approach

Each integrated research team carries out a focused research program containing individual projects tied tightly together by a Team Strategic Plan. Team members, including Team Leaders, are geographically dispersed, remain at their own institutions, and are not grouped at one site. Investigators are invited to become team members after their grant application is selected for NSBRI funding. It is the responsibility of the Team Leader to work with team members, senior management, and NASA to shape an effective team and add value across projects for the purpose of focusing countermeasure research and managing risk in accord with the CPR. Team Leaders play a pivotal role in the Institute's success.

All teams actively communicate among project investigators, foster synergy in experimental activities, share samples, interact with NASA scientists, technical experts, and flight surgeons, and participate in the Institute's Bioinformatics Initiative. (See Table 2 of Appendix IV.) A **Science Integration Manager** is responsible for optimizing scientific collaboration at the NASA/NSBRI interface and establishing ways for NASA scientists, engineers, and computer programmers to work collectively with NSBRI investigators. With advancement through the CRL, team projects interact more closely with JSC medical operations and with the Institute's **User Panel**, made up of current and former astronauts and flight surgeons who evaluate the operational suitability of emerging countermeasures.

Many steps may be required to identify where in the physiological cascade of events to apply countermeasures and what form the countermeasures should take. These steps include performing research to decipher the mechanisms underlying the physiological changes induced in a microgravity environment and preliminary testing of candidate countermeasures on animal or human models of microgravity. An important feature of NSBRI's integrated research team structure is the ability to facilitate, optimize, and support research across teams. For example, dietary and/or immunological countermeasures may be effective in reducing the risk imposed by space radiation, and a combined team approach can address this concept. Another inter-team project may develop new enabling technologies that are lightweight, portable, non-invasive, and unobtrusive, in order to provide in-flight assessment of bone loss and other CSE measures, while also enhancing clinical care. Interdisciplinary efforts in managing risk and countermeasure research are central to the NASA/NSBRI partnership. These efforts span multiple countermeasures, including:

- Training,
- Exercise,
- Diet and nutrition,
- Environmental manipulation,
- Daily rhythm and schedule manipulation,

- Pharmacological agents,
- Selection and retention requirements,
- Monitoring and diagnostic assessment, and
- Medical and surgical procedures.

Space Medicine Research

The Bioastronautics countermeasures development process (Figure 2), medical operations, and the Multilateral Medical Operations Panel are instrumental in countermeasure implementation. NSBRI works closely with NASA space medicine as part of its integrated research team focus on risk management deliverables. The Institute has flight surgeon representation on the teams and receives countermeasures research advice from the User Panel. Also, bidirectional exchanges are encouraged between NSBRI and NASA personnel with respect to evidenced-based space medicine data, the operations environment, engineering and human factors requirements, training, and clinical care.

The Institute also supports targeted projects in space medicine for high-priority operational and clinical status evaluation (CSE) needs. NSBRI scientists, engineers, and clinicians work with their NASA counterparts on specific projects, approved by NASA, related to assessing and ensuring acceptable levels of risk for the human subsystem. NSBRI participation in these joint NASA/NSBRI projects is coordinated through the Office of the Director and supervised by the NSBRI/NASA **Space Medicine Lead/Manager**, who acts as a bridge between NSBRI projects and NASA space medicine. There is coordination among these parallel approaches to advance the field of space medicine, promote more favorable outcomes, and increase the probability that NSBRI countermeasure developments will be successfully used to mitigate health risks in space.

Bioinformatics Initiative

Bioinformatics is a key cross-cutting discipline with sets of analysis tools that span all NSBRI research. The Institute's Bioinformatics Initiative includes data acquisition and mining, modeling, and simulation. The initiative is coordinated by a **Bioinformatics Manager** who works closely with NSBRI and NASA investigators and managers to develop an infrastructure for advanced information techniques, that will effectively catalog data and also assess whether unexpected relationships may be present in acquired databases.

The Institute presently supports the development and implementation of a distributed, but integrated, data management system for ground research, medical, and space flight data. The system, known as the **Institute Data Archive System (IDAS)**, is web-based and will be configured to interface with NASA's archiving systems. All NSBRI investigators are required to electronically transfer appropriate research data to IDAS. A search engine allows NSBRI/NASA researchers and the broader scientific community to access data by categories.

Modeling, simulation, and development of visualization tools are part of the Bioinformatics Initiative designed to integrate research across the different teams and

guide the use of multi-system models. The construction and testing of theoretical models for simulation is complemented by NASA/NSBRI activities in space medicine using hardware analogs, such as a human patient simulator. The coordinated approaches and analysis of complex data apply to all CPR risks and provide a substrate for future refinements in multiple areas, including health care maintenance and support in space, crew selection and rehabilitation, and environmental and engineering adaptations for long-duration space missions.

Evaluation and Metrics

NSBRI, NASA, and periodic external reviews regularly evaluate NSBRI projects, teams, and programs. The Institute's independent, peer-review panels and the BSC provide high quality and objectivity in initial peer review. The BSC, on an annual basis, evaluates the quality, productivity, and progress of each project toward countermeasure development. The BSC also evaluates the teams' annual reports using a set of metrics for assessing team performance over time and across disciplines.

In order to ensure standardized assessment, the Institute, in coordination with NASA, tracks the distribution of projects by CRL and Technology Readiness Level. It also monitors the distribution of tasks and funding by CPR criticality (likelihood and consequences). In consultation with NASA, research projects are prioritized with respect to the CPR and programmatic need. Through its **NSBRI Information Management System (NIMS)**, the Institute has an ongoing electronic database of productivity measures, which includes the number of peer-reviewed publications and invention disclosures/patents per project and team resulting from NSBRI-sponsored research. The system also tracks investigator success in obtaining support for related research aims and objectives from the National Institutes of Health, the Department of Defense, National Science Foundation, Department of Energy, and other funding sources such as the American Cancer Society and American Heart Association.

Productivity metrics and reports from the BSC are provided to the EAC to help determine program effectiveness. The EAC meets semiannually and is charged with assessing strategy and tactical implementation. The Institute Strategic Plan, Team Strategic Plans, competitive proposals, and team accomplishments are discussed with, and vetted through, the EAC. This Council advises the **Director and Board of Directors**, including the **Chairman of the Board and CEO**, regarding the best allocation of resources to meet the Institute's mission, objectives, and goals. The Board of Directors subsequently reviews the major fiscal and programmatic issues confronting the Institute and oversees necessary high-level actions.

The Institute provides NASA with monthly progress and annual scientific and technical reports, along with other documentation of productivity and fiscal accountability. The individual teams and the Institute as a whole are reviewed by NASA in the third or fourth year of each five-year increment.

Program Optimization

Optimization and success of the Institute's Countermeasures Development Research Program rely on the outcome of evaluations and adjustments and/or changes in direction

made by the Institute with its NASA partner. The NSBRI/NASA Steering Committee is an effective mechanism for addressing timely issues to maximize impact, opportunity, and success, given the evolving nature of the CPR and NASA programs (e.g., space flight opportunities, new research initiatives).

Countermeasure Development Research Strategic Timeline

Figure 4 is a five-year strategic timeline with proposed outcomes for NSBRI countermeasure development research.

Figure 4
Countermeasure Development Research Strategic Timeline
FY2003 – FY2007

	2003	2004	2005	2006	2007	Outcome
Priorities and Teams	Support CPR development to prioritize risk for mission scenarios					<ul style="list-style-type: none"> •CPRs and high priority risks established for multiple flight missions •Ownership and continuity throughout the CRLs established for projects addressing specific CQs
	Implement NSBRI/NASA Steering Committee 11 research teams ¹	Develop integrated NASA/NSBRI discipline teams with space medicine input towards high priority research Integrate NSBRI research across Bioastronautics and other NASA programs, including radiation 9 discipline teams integrated with 4 cross-cutting teams ²				
Project Selection	Implement annual ground NRA for CRL 3-7 team research Commencement and growth of NSBRI/NASA space medicine projects 4 flight projects commence at CRL 6-7					<ul style="list-style-type: none"> •Optimal team structure for research •Coordinated selection with NSAs of peer-reviewed translation research projects to develop operationally relevant countermeasures •Directed space medicine research to meet operational needs
	90% CRL 3-5	80% CRL 3-5	30% CRL 5-7	40% CRL 5-7	50% CRL 5-7	
Integrated Program	Extension of NSBRI integrated teams to NASA/NSBRI teams that provide continuity from hypothesis driven countermeasure research to clinical care					<ul style="list-style-type: none"> •Development to CRL/TRL 7 of 20% of funded projects •Development of 5-8 countermeasures with high impact for NASA •Delivery of 3 significant technologies to improve medical care on earth •Delivery of a useful bioinformatics suite of tools for data archiving and manipulation of space biomedical research
		Focused efforts to move CRL 6-7 projects to CEVP Medical technology support program Bioinformatics initiative				
		Expansion of management for NSBRI/NASA research and medical integration and coordination				
Evaluation and Metrics		Peer review and progress evaluations by external panels Progress in managing risk assessed using CPR to answer CQs				<ul style="list-style-type: none"> •Tagging and tracking system for knowledge, IP and deliverable
Programmatic Optimization		Cost/benefit and gap analyses toward countermeasure development performed in coordination with NASA				<ul style="list-style-type: none"> •Demonstration that NSBRI/NASA partnership is effective in generating products to reduce biomedical risk and ensure crew health, safety, and performance
Space Medicine		Countermeasure research and technology development and testing to support CSE Clinical support to medical operations				<ul style="list-style-type: none"> •Provide optimal medical capabilities support to flight surgeons and crews

1 The research teams are Bone Loss; Cardiovascular Alterations, Human Performance Factors, Sleep and Chronobiology; Immunology, Infection and Hematology; Muscle Alterations and Atrophy; Neurobehavioral and Psychosocial Factors; Neurovestibular Adaptation; Nutrition, Physical Fitness and Rehabilitation; Radiation Effects; Smart Medical Systems; and Technology Development.

2 The nine research teams are Bone Loss; Cardiovascular Alterations, Human Performance Factors, Sleep and Chronobiology; Immunology, Infection and Hematology; Muscle Alterations and Atrophy; Neurobehavioral and Psychosocial Factors; Neurovestibular Adaptation; Nutrition, Physical Fitness and Rehabilitation; and Smart Medical Systems the four cross-cutting teams are Radiation Effects, Space Medicine, Technology Development, and Modeling.

JSC/NSBRI Translational Workforce

NASA and NSBRI support the efficient use of all resources in order to obtain maximum scientific results and advances in managing risks on the CPR. With the downsizing of civil servant positions at JSC, the Institute and its NASA partner support a translational workforce of non-government employee scientists and research associates. This workforce is predominantly based at JSC and contributes to the integration and coordination between NSBRI and NASA research efforts. The workforce is linked to Baylor College of Medicine, the lead Consortium institution.

Strategic Relationships

Several programs in the nation are complementary to NSBRI. A number of Federal and non-Federal agencies support pre-clinical research into the mechanisms responsible for microgravity-associated risks and other space flight effects. For example, the National Institutes of Health is the premiere funding agency for the study of bone and muscle abnormalities and various other disease conditions. NSBRI encourages strategic relationships with complementary organizations and programs, to the added benefit of all the involved agencies and parties. Through these relationships, advances in the development of countermeasures and science and health-monitoring technologies will be accelerated. In turn, this will lead to reduction, elimination, or prevention of the adverse consequences of space travel. These discoveries may also be adapted to have practical value in health care delivery on Earth.

The Institute also encourages international relationships, including postdoctoral fellow and visiting scientist exchanges, and research project collaborations with international affiliates. A primary function of national and international strategic relationships is to engage a broad and diverse community of expertise in space life sciences to strengthen and ensure the Institute's success.

Returning Benefits to Earth

Knowledge, discoveries, and technologies developed by the Institute can be adapted for effective diagnosis, therapies, and aids to restore or promote health on Earth. Accordingly, all risk management strategies and results, including medical- and environmental-monitoring deliverables undertaken by the NSBRI, are reviewed monthly. Team Leaders and Institute management discuss promising advances, and NSBRI Headquarters uses NIMS to disseminate information on new discoveries. The Institute is proactive in enabling the return of important scientific and medical findings for Earth-based applications and commercialization.

These activities are complemented by the NSBRI **Industry Forum**, consisting of member representatives of space and biomedical-related industries who keep the Institute in touch with the industrial community. To further promote success in translational research for Earth benefit, the Institute has recently established an advisory **Committee on Research and Technology Transfer** that includes representation from its different advisory panels and boards, as well as outside venture capital experts.

Education, Training, and Public Outreach Program

The goals of this program are:

- Develop education and training programs designed to produce the next generation of space biomedical researchers.
- Transfer the medical and biomedical findings of space research to the scientific community and public.

Most of NSBRI's aims and objectives are pertinent to the Education, Training, and Public Outreach Program.

Scientist Education and Training

NSBRI, through its Consortium institutions and affiliated entities, is strategically positioned to assist NASA in its educational objectives. The Institute offers world-class opportunities for focused biomedical training. The leading professors and outstanding associated institutional resources of NSBRI provide NASA with a unique network for attracting and inspiring exceptional and diverse students and professionals at all levels to pursue unparalleled advanced education and training.

Specific opportunities include:

- **Graduate Student** experiences with access to programs leading to an advanced degree under the supervision of an NSBRI-sponsored investigator at his/her academic institution. Opportunities also exist to participate in university curricula that have space biomedical courses and engage in research activities at NASA laboratories.
- **Postdoctoral Fellowships** for new investigators seeking to pursue a career in space biomedical research. Fellows are encouraged to help bridge the NSBRI/NASA research interface by spending time at both academic and NASA laboratories.
- **Visiting Scientist** placements that provide bilateral exchanges between the academic community and NASA to the benefit of the NSBRI/NASA partnership and the community at large.

K-12 and Undergraduate Education

NSBRI's Education and Public Outreach Team focuses on K-12 and undergraduate education. It is well aligned and coordinated with the Educational Outreach Program of NASA's Office of Biological and Physical Research. The Institute supports peer-reviewed projects that communicate the significance and excitement of space life sciences to local, national, and international audiences, while transferring and disseminating knowledge gained through the biomedical advances achieved by the NSBRI research teams. Going forward the institute's effort in K-12 and undergraduate education will support integrated projects to achieve defined measures of success. Strategic approaches for the team include:

- Enhancing educational access, curricula, and career awareness in space life sciences among K-12 and undergraduate students across diverse communities.
- Designing and conducting teacher professional development programs to help teachers understand space life sciences and improve the learning experiences they provide K-12 and undergraduate students.
- Increasing scientific literacy and public awareness of the real-life impacts of NSBRI research through media, informal science activities, direct mailings, and magazine stories.

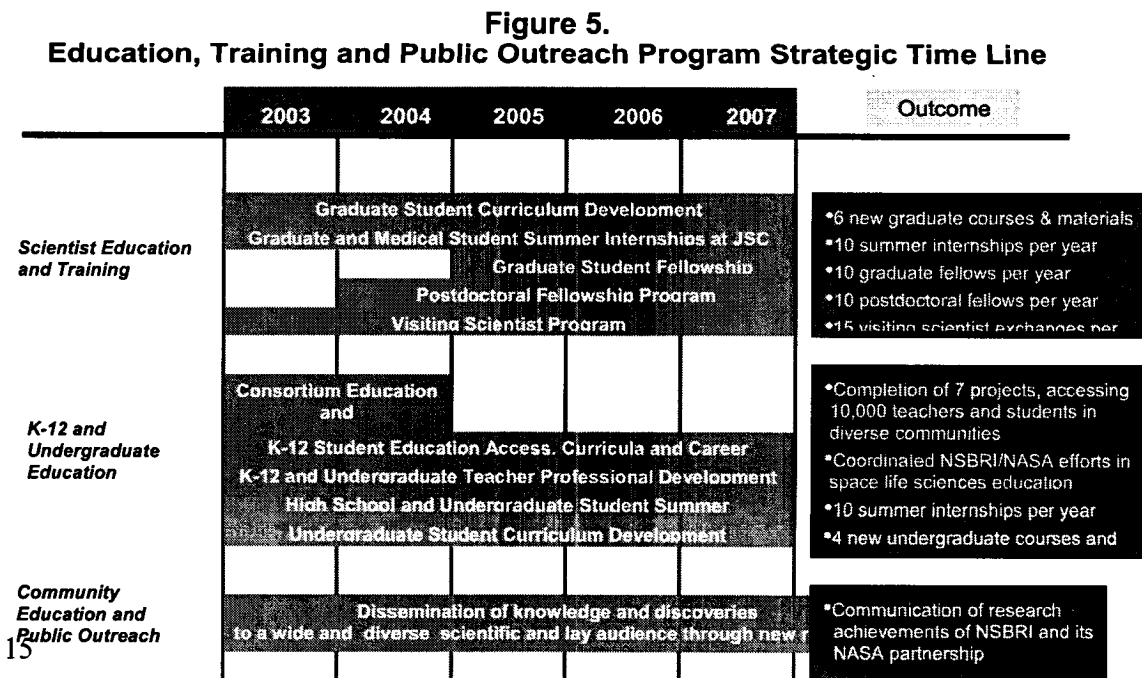
Community Education and Public Outreach

Through public information, the Institute promotes scientific literacy and shares, with a broad audience, an appreciation for the opportunities and the value that space life sciences research offers. This is accomplished in coordination with NASA and academic institutions and uses a multimedia approach orchestrated through NSBRI Headquarters to disseminate biomedical and countermeasures research achievements using:

- An award-winning web site.
- Magazine stories that disseminate space biomedical knowledge.
- Award-winning brochures for public distribution.
- Exhibits for scientific and industrial events.
- Hands-on museum exhibits.
- A network of personal contacts with space and science reporters.
- A national outreach program with PBS and other television exposure.
- Monthly research-based news releases.

Education, Training and Public Outreach Program Strategic Time Line

Figure 5 presents the NSBRI's five year strategic plan for Education, Training and Public Outreach programs as well as proposed outcomes.

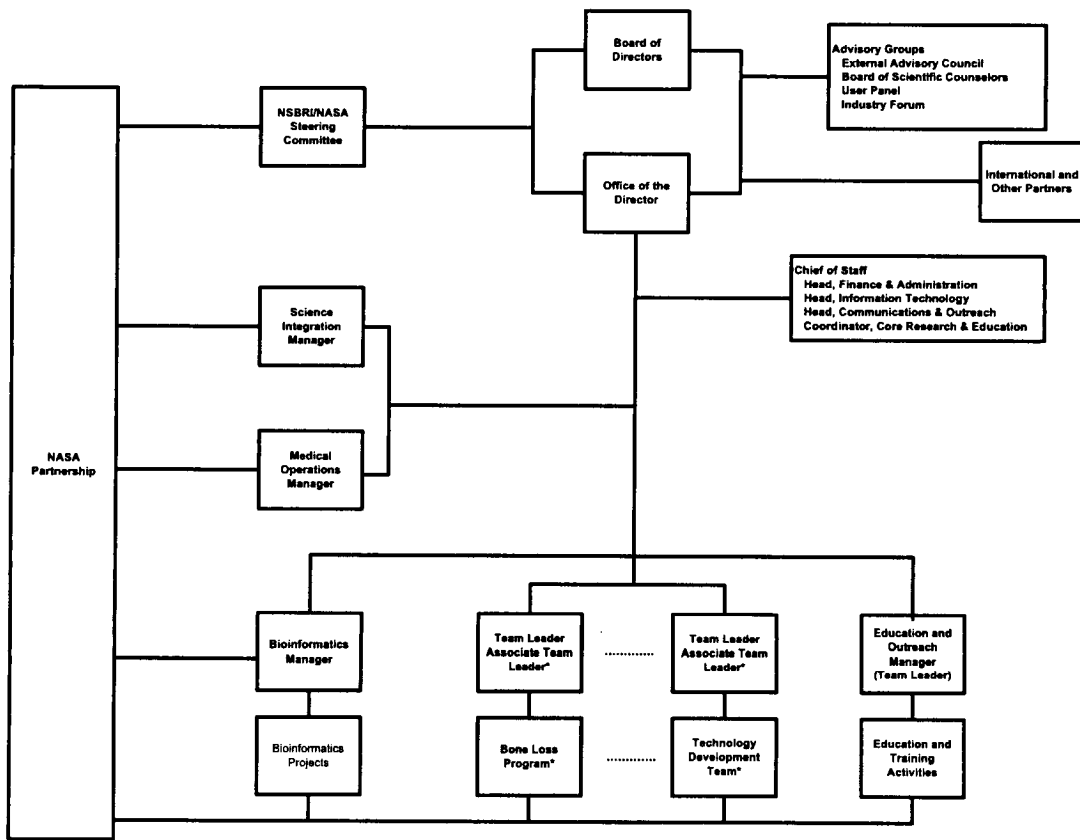


Institute Management

Organizational Structure

NSBRI's organizational structure fosters the NASA/NSBRI partnership at multiple levels. NASA and NSBRI manage and conduct their joint activities to benefit all stakeholders (e.g., NASA, Congress, the public, academic institutions, team leaders, investigators, international partners). The organizational structure facilitates the effective implementation of the Institute's strategies to fulfill its mission, aims, objectives, and goals.

Figure 4. NSBRI Organizational Structure



*Similar Blocks are associated with the other research teams

The Board of Directors has the ultimate authority and responsibility for the activities of the Institute. The Director has the responsibility for successfully carrying out the mission and achieving all of the Institute's aims and objectives. The Associate Director works in the Office of the Director as the deputy. The Chief of Staff oversees operations at NSBRI Headquarters and interfaces with administrative activities at Baylor College of Medicine (as the lead Consortium institution) and at JSC. The Science Integration Manager and the Space Medicine Lead/Manager facilitate, coordinate, and maximize interactions among NSBRI investigators and NASA scientists, engineers, flight surgeons, and astronauts to develop productive countermeasures. The Team Leaders manage their respective integrated programs in research and education. There is also a Bioinformatics Manager who implements the Institute's Bioinformatics Initiative. The NSBRI's advisory system includes the BSC, EAC, User Panel, and Industry Forum.

Budget

The Institute's core budget is established by NASA and supports a critical mass of peer-reviewed research activity that uniquely and effectively contributes to NASA's Bioastronautics Strategy. NASA and NSBRI have agreed that either entity can propose specific work element/projects that are within the overall scope of the NASA Cooperative Agreement Notice, but by their nature would not normally be research activities included within the NSBRI core research plan. When such projects are approved, a project plan for implementation will be prepared and included as a supplement to the Cooperative Agreement.

Correspondence with the President's Management Agenda

NSBRI is an exemplary, performance-driven science institute. Its partnership with NASA addresses all five components of the President's Management Agenda.

Strategic Management of Human Capital – NSBRI is a private, non-profit entity that engages outstanding biomedical researchers to work with, and fill skill shortages at, NASA augmenting government civil servants with the unique and complimentary NSBRI skill base.

Competitive Sourcing – NSBRI, competitively supported by NASA, implements aims, objectives, and goals that focus on high-quality, timely results and outcomes desired by NASA.

Improved Financial Performance – NSBRI uses a streamlined accounting process operating with minimal bureaucracy and provides NASA with accurate and timely financial reports and clean audits. The NSBRI financial management system allows tracking of resources as well as day-to-day operations and task-completion productivity metrics.

Expanded Electronic Government – NSBRI's distributed structure and its multiple joint interactions with NASA are supported by efficient electronic communications, business operations, and data management capabilities (e.g., NIMS, IDAS). Most business operations, including grant proposal submission and productivity monitoring, are conducted electronically.

Budget and Performance Integration – NSBRI is task-oriented and uses the CPR, in coordination with NASA, for research prioritization and resource allocation enabling the development of countermeasures. There is a focus on deliverables, progress, productivity metrics, and a process for shifting resources to meet risk management needs.

Appendix I

The 55 Risks of the Bioastronautics Critical Path Roadmap

ID ¹	Risk Title	Rank ²	Discipline Area ³
1	Inability to Maintain Acceptable Atmosphere in Habitable Areas	1	Advanced Life Support
2	Inability to Provide and Recover Potable Water	2	Advanced Life Support
3	Inadequate Supplies (including maintenance, emergency provisions, and edible food)	2	Advanced Life Support
4	Inability to Maintain Thermal Balance in Habitable Areas	3	Advanced Life Support
5	Inability to Adequately Process Solid Wastes	3	Advanced Life Support
6	Inadequate Stowage and Disposal Facilities for Solid and Liquid Trash Generated During Mission	4	Advanced Life Support
7	Inadequate Nutrition (Malnutrition)	1	Food & Nutrition
8	Unsafe Food Systems	2	Food & Nutrition
9	Acceleration of Age-Related Osteoporosis	1	Bone Loss
10	Fracture & Impaired Fracture Healing	2	Bone Loss
11	Injury to Soft Connective Tissue, Joint Cartilage, & Intervertebral Disc Rupture w/ or w/o Neurological Complications	3	Bone Loss
12	Renal Stone Formation	4	Bone Loss
13	Occurrence of Serious Cardiac Dysrhythmias	1	Cardiovascular Alterations
14	Impaired Response to Orthostatic Stress	1	Cardiovascular Alterations
15	Diminished Cardiac Function	2	Cardiovascular Alterations
16	Manifestation of Previously Asymptomatic Cardiovascular Disease	3	Cardiovascular Alterations
17	Impaired Cardiovascular Response to Exercise Stress	4	Cardiovascular Alterations
18	Human Performance Failure Because of Poor Psychosocial Adaptation	1	Human Behavior & Performance
19	Human Performance Failure Because of Sleep and Circadian Rhythm Problems	2	Human Behavior & Performance

20	Human Performance Failure Because of Human System Interface Problems & Ineffective Habitat, Equipment, Design, Workload, or Inflight Information and Training Systems	3	Human Behavior & Performance
21	Human Performance Failure Because of Neurobehavioral Dysfunction	4	Human Behavior & Performance
22	Immunodeficiency/Infections	1	Immunology, Infection & Hematology
23	Carcinogenesis Caused by Immune System Changes	1	Immunology, Infection & Hematology
24	Altered Hemodynamic and Cardiovascular Dynamics caused by Altered Blood Components	1	Immunology, Infection & Hematology
25	Altered Wound Healing	2	Immunology, Infection & Hematology
26	Altered Host-Microbial Interactions	3	Immunology, Infection & Hematology
27	Allergies and Hypersensitivity Reactions	3	Immunology, Infection & Hematology
28	Loss of Skeletal Muscle Mass, Strength, and/or Endurance	1	Muscle Alterations & Atrophy
29	Inability to Adequately Perform Tasks Due to Motor Performance, Muscle Endurance, and Disruption in Structural and Functional Properties of Soft & Hard Connective Tissues of the Axial Skeleton	1	Muscle Alterations & Atrophy
30	Inability to Sustain Muscle Performance Levels to Meet Demands of Performing Activities of Varying Intensities	2	Muscle Alterations & Atrophy
31	Propensity to Develop Muscle Injury, Connective Tissue Dysfunction, and Bone Fractures Due to Deficiencies in Motor Skill, Muscle Strength and Muscular Fatigue	3	Muscle Alterations & Atrophy
32	Impact of Deficits in Skeletal Muscle Structure and Function on Other Systems	NR	Muscle Alterations & Atrophy
33	Disorientation and Inability to Perform Landing, Egress, or Other Physical Tasks, Especially During/After G-Level Changes (Acute spontaneous & provoked vertigo, nystagmus, oscillopsia, poor dynamic visual acuity)	1	Neurovestibular Adaptation

34	Impaired Neuromuscular Coordination and/or Strength (Gait ataxia, postural instability)	2	Neurovestibular Adaptation
35	Impaired Cognitive and/or Physical Performance Due to Motion Sickness Symptoms or Treatments, Especially During/After G-Level Changes (Including short term memory loss, reaction time increase, drowsiness, fatigue, torpor, irritability, ketosis)	3	Neurovestibular Adaptation
36	Vestibular Contribution to Cardiorespiratory Dysfunction (Postlanding orthostatic intolerance, sleep and mood changes)	4	Neurovestibular Adaptation
37	Possible Chronic Impairment of Orientation or Balance Function Due to Microgravity or Radiation (Imbalance, gait ataxia, vertigo, chronic vestibular insufficiency, poor dynamic visual acuity)	5	Neurovestibular Adaptation
38	Carcinogenesis Caused by Radiation	1	Radiation Effects
39	Late Degenerative Tissue Effects including Non-Cancer Mortality, Cataracts, and Central Nervous System (CNS) Effects	2	Radiation Effects
40	Synergistic Effects from Exposure to Radiation, Microgravity and other Spacecraft Environmental Factors	3	Radiation Effects
41	Early or Acute Effects from Radiation Exposure	4	Radiation Effects
42	Radiation Effects on Fertility, Sterility, and Heredity	5	Radiation Effects
43	Trauma and Acute Medical Problems	1	Clinical Capabilities
44	Toxic Exposure	2	Clinical Capabilities
45	Altered Pharmacodynamics and Adverse Drug Reactions	3	Clinical Capabilities
46	Illness and Ambulatory Health Problems	4	Clinical Capabilities
47	Prevention, Development and Treatment of Space-Induced Decompression Sickness	5	Clinical Capabilities
48	Difficulty of Rehabilitation Following Landing	6	Clinical Capabilities
49	Post-landing Alterations in Various Systems Resulting in Severe Performance Decrements and Injuries	1	Multisystem (Cross Risk) Alterations

50	Allergies and Hypersensitivity Reactions from Exposure to the Enclosed Spacecraft & Other Environmental Factors	3	Environmental Health
51	Inability to Maintain Acceptable Atmosphere in Habitable Areas Due to Environmental Health Contaminants	1	Environmental Health
52	Inability to Provide and Recover Potable Water Due to Environmental Health Contaminants	2	Environmental Health
53	Inadequate Nutrition (Malnutrition) Due to Inability to Provide and Maintain a Bioregenerative System	3	Advanced Life Support
54	Difficulty of Rehabilitation Following Landing Due to Nutritional Deficiencies	4	Food & Nutrition
55	Human Performance Failure Due to Nutritional Deficiencies	3	Food & Nutrition

¹Risk Identification number: Unique number assigned to each risk (1-55) used to track/identify each risk.

²The Rank Order assigned to each risk by discipline experts in each Discipline; a Discipline may have more than 1 risk with the same risk ranking.

³There are 12 Discipline Areas in the CPR.

Appendix II

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE POLICY ON TEAM LEADERSHIP

I. Overview

Each Institute team is led by a single Team Leader who is assisted by an Associate Team Leader. Team Leaders play a pivotal role in guiding the Institute's research program and achieving the ultimate success of the Institute. Their expertise and "hands-on" approach to research management add value across projects and across teams. The Team Leader is guided by the Bioastronautics Critical Path Roadmap (CPR), which is the cornerstone for developing the team's integrated strategic research plan, the key to accomplishing the Institute's mission. The Team Leader's stature and reputation as a strong scientist enable recruitment of other scientific leaders as team members. The Team Leader's communication skills and insight enable the appropriate synergistic discussions among the various research projects, with the objective of assuring a team research program that has higher value than the sum of the values of its separate projects.

II. Duties and Responsibilities

Team Leaders are responsible for:

- Preparing and periodically updating the team research strategic plan. This plan should be consistent with the Institute mission, the CPR and available resources.
- Reporting progress to the Institute's External Advisory Council (EAC), Board of Scientific Counselors (BSC) and NSBRI management.
- Preparing and presenting the initial recommendation to the EAC of new research projects for inclusion in the team's program.
- Representing the team and disseminating knowledge about team activities and progress to NASA; specifically coordinating with NASA-JSC scientists and physicians, the scientific community and the general public.
- Pursuing involvement with NASA operational activities.
- Maintaining appropriate communication links among the team investigators and to other team leaders.
- Developing, with team investigators, individual project plans that ensure scientific and operational synergy and lead to productive countermeasure development.
- Nurturing opportunities and seeking funding support to collaborate with, and cross-fertilize, research within and between NSBRI teams and with Johnson Space Center, other NASA Centers and other agencies.
- Acting as the senior NSBRI discipline representative for ongoing development of the CPR.

Associate Team Leaders assist Team Leaders in carrying out the above activities.

III. Qualifications

Team Leaders are NSBRI-funded principal investigators who possess the following qualifications:

- Achieved intermediate or senior rank at a research or educational institution.

- Demonstrated record of securing independent competitive research funding for at least the last five years.
- Recognized within the biomedical community as an outstanding research contributor to at least one field of study; prior involvement with a NASA flight investigation would be beneficial.
- Manifest broad scientific understanding across the team's research area.
- Demonstrated leadership and program/group management skills, as evidenced by experiences such as a section head, department chair, dean, research center director or principal investigator on a program project.
- Exhibit good communication, public speaking and organizational skills.
- Show a willingness and availability to spend the necessary time and energy to fulfill the role of Team Leader.

Associate Team Leaders are principal or co-investigators on NSBRI-funded projects who possess at least the first four of the above qualifications required for a Team Leader. Generally, Team Leaders and Associate Team Leaders are not from the same institution.

IV. Term of Service

Team Leaders are appointed by the Director for a term that is identical with the term of their NSBRI-funded research project (generally four years), subject to satisfactory performance as determined at their annual performance review. The Team Leader appoints Associate Team Leaders for a term that does not exceed the Team Leader's term of service. The Team Leader's term is competitively renewable, while the Associate Team Leader's term is renewable.

V. Funding and Authority

Team Leaders and Associate Team Leaders are provided with discretionary funds to enable them to carry out their duties and responsibilities. Wide latitude is provided concerning the expenditure of these funds within the guidelines of the involved institutions. Such funds may be used for support personnel, team meetings, special travel and other expenses generally associated with team communication and operations. However, these funds may not be used to support research.

Team Leaders are ultimately responsible for carrying out the duties and responsibilities listed in Section II. They are expected to work cooperatively with their Associate Team Leader in all matters and should develop a clear understanding of the distribution of their shared responsibilities. Team Leaders report to the Director.

VI. Selection

In the year before a Team Leader's term of service ends, a special "Call for Candidates" section will be included in the annual Institute Research Announcement requesting applications for the Team Leader's position. Research applications may then be accompanied by a special short application for the Team Leader position. Following the evaluation of the research application by a peer committee, Institute Senior Management (Director and Associate Director) will evaluate the merits of the applicants for Team

Leader and recommend a selection to the Chairman of the Board who will seek confirmation of the selection from the Board of Directors.

Associate Team Leaders are nominated and selected by the Team Leader, with the advice and consent of the Director in consultation with the Associate Director.

In selection of Team Leaders and Associate Team Leaders, attempts will be made to balance the scientific and managerial expertise of candidates and to develop diversity within the Institute's research leadership.

VII. Training and Support

To assist Team Leaders in performing their duties, the NSBRI provides electronic reporting and managerial tools, along with training as needed. Forums are held at least three times a year for Team Leaders to meet as a group with the Director and Associate Director.

VIII. Performance Evaluation

Once a Team Leader is selected, five groups evaluate the performance and effectiveness of Team Leaders: the EAC, BSC, team principal investigators, NSBRI Senior Management and NASA. Each group focuses on different aspects of a Team Leader's performance:

- Annually, the BSC will review each team's annual report of productivity and progress in carrying out the team strategy, including evidence that the research projects are functioning synergistically within the research team and evidence that the team is collaborating effectively with other teams and with NASA life scientists.
- Semi-annually, the EAC will review the effectiveness of the Team Leader in communicating the team vision and successes, and in discussing and handling team issues and problems.
- Annually, team principal investigators will evaluate the leadership, communication and other relevant skills of their Team Leader.
- Annually, Institute Senior Management will evaluate the Team Leader's overall effectiveness and responsiveness.
- At least every four years, and more frequently if necessary, the four research area representatives on the EAC and BSC (two each) will review the team strategic plan and furnish a written critique of the strengths and weaknesses of the plan along with a rating of the overall team strategy embedded in the plan.
- Every five years, just prior to conducting an Institute-wide review, an *ad hoc* review team, appointed by NASA, will evaluate all aspects of the team's performance, including the Team Leader's performance.

Institute Senior Management will produce an annual overall rating of each Team Leader's performance based on the available inputs.

An unsatisfactory Team Leader rating will normally result in a specific warning to the Team Leader and include a recommended action plan to correct the identified deficiencies in performance. Two unsatisfactory Team Leader ratings in successive years

will result in removal of the Team Leader and appointment of an acting Team Leader to serve out the remainder of the Team Leader's term. The Institute supports the need for leadership continuity, but only if the evaluative process supports an annual reappointment. Team Leaders are ultimately judged by their team's ability to successfully develop and deliver, in whole or part, countermeasures in areas of high impact for NASA, for the purpose of decreasing the biomedical or human performance risks associated with long duration human space flight.

Associate Team Leaders are evaluated annually by Team Leaders for their contribution to team goals, achievements, function, productivity and representation. Unsatisfactory performance may lead to removal of Associate Team Leaders, but such action requires the concurrence of the Director in consultation with the Associate Director.

IX. Conflict of Interest

Team and Associate Team Leaders must adhere to the highest ethical standards as they carry out their leadership duties. They must not make decisions based on institutional affiliation or personal bias. They must conduct all leadership duties with integrity, fairness and objectivity to ensure the scientific credibility of the Institute.

To avoid a conflict of interest during a selection in which a Team Leader has a competing application, Institute Senior Management selects the Team Leader and project before any other projects are selected. Then the Team Leader develops and presents a selection recommendation concerning the other competing projects to the EAC. The EAC recommends the final selection to Institute Senior Management, taking into account the science merit rating and programmatic relevance rating furnished by the BSC in addition to the Team Leader recommendation. Institute Senior Management makes the final selection decisions following coordination with NASA. If the Team Leader does not have a competing application during a selection cycle, the process is similar but the Team Leader will be assisted by the Associate Team Leader in developing the selection recommendation to the EAC.

Appendix III

Mapping Between NSBRI Research Teams and CPR Risks

RESEARCH TEAM	NUMBER OF UNIQUE RISKS	CPR RISK NUMBERS
Bone Loss	4	9, 10, 11, 12
CV Alterations	5	13, 14, 15, 16, 17
Human Perf Factors	1	19
Imm, Inf & Hem	5	22, 23, 25, 26, 27
Muscle Alt & Atrophy	5	28, 29, 30, 31, 32
Neurobehav, Psychosocial	3	18, 20, 21
Neurovestibular Adaptation	5	33, 34, 35, 36, 37
Nut, Phys Fitness & Rehab	3	7, 54, 55
Radiation Effects	5	38, 39, 40, 41, 42
Smart Med Systems	6	43, 44, 45, 46, 47, 48
Tech Development	N/A	
	1	49 (cross-risk)
TOTAL	43	

Appendix IV

Table 1. Example of Project Research Activities from Team Strategic Plans
(Cardiovascular Alterations Team)

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
COHEN/Effects of Space Flight on Cardiovascular Stability	Orthostatic hypotension Exercise Dysrhythmias	<ul style="list-style-type: none"> Pharmacological (Midodrine and Spironolactone) Diet (Electrolytes) 	Pre and post flight humans	Effects of microgravity on: <ul style="list-style-type: none"> CV regulation (CV System identification) Dysrhythmias (T-Wave Alternans) 	Pre and post flight in humans of <ul style="list-style-type: none"> Midodrine Spironolactone Diet (Electrolytes) 	Pre and post flight in humans of <ul style="list-style-type: none"> Midodrine Spironolactone Diet (Electrolytes) Spironolactone
COOLAHAN/Distributed Simulation of Integrated Human Function	Orthostatic hypotension Dysrhythmias Cardiac function CV Disease Exercise	Simulation of effects of wide variety of different potential countermeasures including exercise	Computer model of the cardiovascular system integrated with other systems	Develop and validate accurate model of myocyte and heart	<ul style="list-style-type: none"> Develop integrated CV model Develop integrated model incorporating other systems Simulate effects of space flight and potential CMs 	Analyze data from animal and human tests of countermeasures
DELP/Circulatory Remodeling with Simulated Microgravity	Orthostatic hypotension Cardiac function Exercise	Peripheral vascular countermeasures	<ul style="list-style-type: none"> Hindlimb unloading (HU) of rats Shuttle flight (STS-107) of rats 	Measure effects and mechanisms of HU and shuttle flight on: cerebral & peripheral vascular beds, lymphatics, cardiac mass	Evaluate data to identify and then test potential countermeasures	Test countermeasures in human studies

Table 2. Example of Project Integration Activities from Team Strategic Plans
(Smart Medical Systems Team)

	CRUM HIFU for mission critical care	DAVIES Vascular genomics in g transitions	KLEMPNER Smart microorganism detection sys	PUTCHA Microcapsule drug formulation	SOLLER Non-invasive blood & tissue chemistry	SUTTON Near infrared brain imaging for space med	THOMAS Diagnostic 3D ultrasound for space	THOMAS Echocardiographic resource
Internal Communication	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings	Monthly team telecon; Biannual team meeting; NSBRI retreat; Biannual NASA meeting; National scientific meetings
Integrated Experiment Development	Sensor aspects link to both THOMAS projects	Vascular studies link Cardiovascular Alterations team	Algorithms link to SUTTON project; Phage library studies link to ITH team	Promethazine development links to Neurovestibular Adaptation team	Sensor links to SUTTON project; Applications links to Muscle team	Sensor links to SOLLER project; Algorithms link to KLEMPNER and THOMAS projects	Sensor links to CRUM project; Algorithms link to SUTTON project; Echo links to CV team	Sensor links to CRUM project; Algorithms link to SUTTON project; Echo links to CV team
Sample Sharing			Specimen sharing with JSC microbiology		Collaboration with CABRARA project	Cognitive task sharing with JSC flight surgeons	Vascular investigations link to DAVIES project	Resource is shared within NSBRI and NASA
Synergistic Studies of Opportunity	Studies with DoD/DARPA	Bioengineering Center, U Penn	SRU Biosystems, biotechnology industry	Pharma industry collaborations	Medical assessment for diabetics, surgical patients	Neuro monitoring in ICU, Harvard	Cardiac, renal imaging apps in med practice; flight apps	Resource of National value
Development of Computer Model of Integrated Human Function	Image / model guided surgery	Data mining	Neural nets for pattern recognition		Algorithms for pattern identification	Neural nets, software engineering platform	Algorithms for individualized astronaut models	Modeling for training purposes

Acronym List

Acronym	Definition
CPR	Bioastronautics Critical Path Roadmap
BSC	Board of Scientific Counselors
CAMP	Cooperative Agreement Management Plan
CRL	Countermeasure Readiness Levels
CSE	Clinical Status Evaluation
EAC	External Advisory Council
IDAS	Institute Data Archive System
JSC	Johnson Space Center
NASA	National Aeronautics and Space Administration
NIMS	NSBRI Information Management System
NRA	NASA Research Announcement
NSBRI	National Space Biomedical Research Institute

Appendix B

Cooperative Agreement
Management Plan

National Space Biomedical Research Institute
(NSBRI)

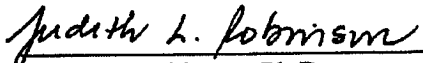
June 2003

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
JOHNSON SPACE CENTER
SPACE AND LIFE SCIENCES DIRECTORATE
HOUSTON, TEXAS 77058

Approval



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Version and Change Record

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Revision A- June 27, 2003

1. Scope

This document is a management plan (Cooperative Agreement Management Plan - CAMP) referenced in the NASA Johnson Space Center/National Space Biomedical Research Institute (NSBRI) Cooperative Agreement # NCC 9-58 (CA). This document provides details through which NASA and the NSBRI will manage and conduct their joint activities in support of the NASA Bioastronautics effort. The NASA Contracting Officer's Technical Representative (COTR) and the NSBRI's Chairman of the Board (Chairman) control changes to this document. No agreements in this document shall extend beyond the boundaries defined in the CA. In the event of ambiguity between the CA and the CAMP, the CA takes precedence.

2. General

The NSBRI is a Texas not-for-profit corporation, whose sole member is Baylor College of Medicine. This effort is the result of a competitive solicitation by NASA through Cooperative Agreement Notice 9-CAN-96-01 (CAN), dated June 10, 1996, with selection by the NASA Associate Administrator for the Office of Life and Microgravity Sciences and Applications (OLMSA) occurring on March 14, 1997. Continuation of the NSBRI effort for the second 5-year period of performance, was endorsed by the NASA Chief Scientist's review November 28- December 2, 2000 and by the Vaitukaitis NSBRI Strategic Plan Review held on June 10-11, 2002. In the remainder of this document, these entities will be referred to as NASA and NSBRI. The relationship between NASA and NSBRI is defined in the CA and the CAMP. This effort is planned to be a long-term (20 year) activity.

NASA is attempting to identify, implement, and test new ways of doing business that will achieve the desired end products with an optimized expenditure of resources (time, money, facilities, and personnel). Through implementation of this document, the NSBRI agrees to partner with NASA to identify and test new ways of doing business. All such resource expenditures (as identified above) shall be challenged by both parties to ensure that such resources are value added and/or are required by law, procurement policies, or prudent fiscal stewardship. The NASA/NSBRI partnership established and implemented through this CA/CAMP shares the joint objective of forging new relationships between government and academia, not-for-profit, and commercial entities, resulting in a new model for NASA to use in developing additional "institutes" or for other government agencies to use for similar purposes.

While the CA is between the Johnson Space Center's Space and Life Sciences Directorate and the NSBRI, NASA Headquarters and other NASA centers will be involved in various aspects of planning, support, and implementation of this effort. Headquarters activities

associated with the NSBRI are related to oversight guidelines and policies and budget allocation.

3. Guiding Policy

Pursuant to the Cooperative Agreement, NASA and NSBRI will have continuous significant interaction and pursue the Mission and Objectives that have been specified by NASA. The NSBRI's Mission, as stated in the CAN, is as follows:

“The Mission of the Institute will be to lead a National effort for accomplishing the integrated, critical path, biomedical research necessary to support the long term human presence, development, and exploration of space and to enhance life on Earth by applying the resultant advances in human knowledge and technology acquired through living and working in space. The Institute will be the focal point of NASA sponsored space biomedical research.”

The following are the stated objectives that will be implemented through the CA/CAMP:

- Integrate the knowledge base relevant to the biomedical response of humans in space. This includes understanding and quantifying, to the extent possible, the risk levels associated with this knowledge base, and recommending acceptable risk levels for long-duration missions. Risk levels in this context relate to both present and future medical risk to the human participants as a result of deleterious effects of space flight, as well as the subsequent risk to overall mission success.
- Develop and manage the implementation of an integrated research plan that will develop the required knowledge and technologies (across all biomedical and associated technological disciplines) to enable long-duration human space flight, including specific countermeasures where required.
- Develop and provide a science management process that will support the overall human in space biomedical research program.
- Demonstrate an understanding of the space medicine environment through an integrated on-site presence at JSC; feedback this knowledge to the discipline research teams.
- Ensure the dissemination of advances in knowledge resulting from this program to the scientific community.
- Facilitate science community access to the NASA space infrastructure associated with biomedical research.
- Promote and provide active collaboration with for-profit entities to ensure that developed technologies are transferred to the private sector.
- Implement a “best value” research program for the available resources.
- Conduct education and public outreach programs consistent with the NSBRI's Mission and in support of NASA's education and public outreach objectives.

Both NASA and the NSBRI bring significant assets to this Mission and set of Objectives. An essential aspect of the CA/CAMP is that these assets must be synergistically blended to become significantly more productive than either working alone. The CA/CAMP will attempt to identify top-level guidelines and policies to enable this interaction without creating unnecessary constraints; therefore, this CA/CAMP is intentionally broad based and top level. NASA and NSBRI are expected to be innovative while remaining within the boundaries of legal and procurement policies.

4. NSBRI Proposal

The NSBRI proposal as submitted in response to the CAN was formally evaluated and accepted by NASA. It served as the baseline through which the NSBRI operations and processes were developed. The updated NSBRI Strategic Plan (the draft developed and submitted on May 24, 2002) supplants the NSBRI proposal and forms the new baseline document. The new baseline includes the substantive responses to recommendations by the Strategic Plan Review Committee (June 21, 2002).

NSBRI shall notify NASA and request formal written concurrence for any proposed changes to this baseline when such changes would significantly impact the mission, objectives or methods of business. Significant impact in this context means that a group of qualified external reviewers knowing the relevant information would deem such changes significant and of importance to the NSBRI and NASA for overall execution of the CA/CAMP.

5. Metrics

In the future, NASA will quantitatively assess the progress of the NSBRI, utilizing agreed upon metrics for analysis of deliverables. Such metrics shall be reported in a quarterly basis. The agreed upon metrics will demonstrate NSBRI performance within the framework of the Bioastronautics goals and objectives, as well as NSBRI goals and objectives. Metrics will be used to assess NSBRI efforts from multiple perspectives including risks and associated critical questions, progress by scientific discipline, as well as deliverables and outcomes. The agreed upon metrics will address effectiveness in terms of risk reduction, increased efficiency and Earth-based benefits.

6. Bioastronautics Strategy

The NASA Bioastronautics effort is sponsored by the Office of Space Flight, the Office of Biological and Physical Research, and the Office of the Chief Health and Medical Officer. The NASA/NSBRI partnership is an important Bioastronautics partnership that is instrumental in enabling NASA to reach its vision and accomplish its goals. Bioastronautics is a focused effort to enable human exploration of space through effective risk management solutions and innovative science and technology discoveries. Bioastronautics steadfastly ensures the safety, health, and productivity of space crews, and utilizes the knowledge gained in such a quest to improve life on Earth.

The Bioastronautics risk-based approach allows for understanding and controlling the human health and performance risks, identifies specific outcomes, enables informed decision making, and provides the Agency with highly developed risk management solutions to meet its enabling and mission-driven goals and objectives. These goals and objectives foster a set of mission requirements that define the Bioastronautics risk management research and technology program, including the astronaut health care system. Within the context of the NASA Bioastronautics Strategy, the NSBRI is a major stakeholder in the focused biomedical research effort for risk management purposes, including countermeasure definition and development; supporting mission critical issues for human space flight; disseminating & archiving data; and performing advocacy/development of the space biomedical research community.

7. Special Emphasis Areas

The following areas and activities require additional guidance for ensuring successful NASA/NSBRI interaction. These guidelines should not be regarded as a contractual policy but they indicate the current NASA/NSBRI approach for ensuring that important component processes will occur. Joint ownership and evolution of the Bioastronautics Critical Path Research Plan as a strategic document for guiding research is considered mandatory.

7.1 Maintaining Continued Scientific Interaction Between NASA and NSBRI Research Teams

NASA and NSBRI have agreed that attaining both the short-term and long-term objectives for biomedical research and technology activities within the framework of the CA/CAMP will require a fully integrated team approach. The ultimate goal is that investigators from these teams develop collegial relationships leading to joint research efforts (supported by any available funding source). It should be noted this cannot and will not be a directed outcome but one that will be facilitated to the maximum extent by NASA and NSBRI. When joint projects are not appropriate or desirable for sound reasons, in order to avoid duplicative efforts, the minimally acceptable approach is that investigators inform their joint discipline team of their intended research and proposal thrusts prior to proposal submission. It is equally important that NSBRI clinicians are identified and that they become involved with their NASA Space Medicine counterparts to jointly resolve clinical issues, as appropriate.

In order to provide this facilitation and integration, both NASA and NSBRI will have a designated team leader for each of the research areas (Human Performance Factors, Sleep, and Chronobiology; Neurovestibular Adaptation; Cardiovascular Alterations; Muscle Alterations and Atrophy; Bone Loss; Immunology, Infection and Hematology; Technology Development; Neurobehavioral and Psychosocial Factors; Nutrition, Physical Fitness and Rehabilitation; Smart Medical Systems; and Education & Public Outreach). NSBRI will have coordination responsibility for selected areas within Radiation Interaction Effects. These leaders will communicate regularly, and should have formal telecons (and face-to-face meetings when appropriate) on a monthly basis. The minutes from these communications should be jointly developed and concurred upon by the team leaders with copies provided to the COTR and NSBRI Director. Space Medicine

activities require comparable interactions; NASA and NSBRI will support additional communication, travel to laboratories, or other types of research and resource sharing to the maximum extent possible. NSBRI research teams will have an on-site presence at JSC, integrated into the research activities and space medicine efforts to facilitate an operationally focused research program.

NASA and NSBRI will maintain a joint schedule of events and activities which cross-utilize NASA/NSBRI assets; this plan will be maintained by NASA and updated as required.

NASA and NSBRI have agreed to conduct joint workshops on a biennial basis. These NASA/NSBRI workshops serve as a collaborative forum to review, analyze, coordinate, and integrate research results and clinical observations as well as progress within and across all disciplines. These workshops will nominally alternate with a biennial symposium to be hosted by NASA, during which all NASA supported biomedical research will be presented.

While the Mission of the NSBRI is to lead the National effort in space biomedical research, NASA is committed to maintaining a strong, openly competitive, peer reviewed program comprising individual investigator awards, center awards (e.g. Radiation NSCOR), and various interagency activities. NASA and the NSBRI will develop procedures for monitoring and policies for appropriate action should the current balance between NSBRI and non-NSBRI awards require adjustment. However, there is no specific limitation to the percentage of total Code UB research funding that may be applied to the NSBRI research program.

As a further extension of the guidelines identified in the section, NASA and NSBRI agree to develop a joint, robust, and continual process that reviews, consolidates, and coordinates results, both within disciplines and between disciplines. Depending upon the approaches agreed upon between NASA and NSBRI, this activity may be identified as a separate project.

7.2 JSC Provided Assets

For its part of the CA/CAMP, NASA/JSC will provide the following assets (as related to the content of the CA/CAMP) on a non-interference basis:

- Facilitation of open access to all civil servants engaged in activities related to the content of the CA/CAMP.
- Access to all ground-based research laboratories and instrumentation. NASA will maintain the function and calibration of any associated instrumentation. The NSBRI will supply the needed resources (personnel, instrumentation, expendables, ancillaries, pro rata) that are required to execute any NSBRI particular project within NASA facilities.

- Notification of and invitation to NASA meetings, symposia, specialized/selected training, and other activities that would benefit the execution of the CA/CAMP.
- Access to specialized data/information (with any required procedures for maintaining NASA policy and subject confidentiality, when appropriate, including clinical and research data) that may not be publicly available, when such data/information are deemed essential by NASA/NSBRI for the NSBRI to fulfill its Mission and Objectives.
- Coordinated and facilitated access to project scientists, managers, physicians and engineers.
- Coordination/facilitation for identification and utilization of any non-JSC NASA research assets that may be located at other NASA centers.
- Coordinate/facilitate NASA research team access to NSBRI consortium laboratories
- Facilitate access to space flight when such access is deemed critical to the accomplishment of the CA/CAMP.

7.3 NASA/JSC Intramural Effort

The specific role of the NSBRI is to provide senior scientific personnel resources that add value to the overall JSC intramural research efforts in a cost-effective manner. Funding will be the responsibility of the individual NASA principal investigators.

Within this context, NASA and NSBRI have agreed to the following:

- The long-term goal/desire as stated in Section 7.1 above is that the NASA intramural and NSBRI researchers develop collegial, joint proposals and research efforts. Such proposals could be submitted in response to NASA open NASA Research Announcement (NRA) solicitations, to NSBRI solicitations, or to solicitations of other funding agencies. Resource requirements for joint activities will be defined in the proposal. NASA will provide direct funding to NSBRI (over and above core funding when a NASA NRA selection occurs) for any successful, peer-reviewed activities within this category.
- For currently approved JSC intramural research efforts, the NSBRI will review each approved research activity, and will make recommendations to NASA on how the NSBRI could contribute to the effective implementation of that activity. The joint objective of NASA and NSBRI is the efficient utilization of all resources in order to obtain the maximum scientific results for the resources available. NSBRI shall provide senior independent investigator level resources required for JSC intramural research only through academic organizations. The use of NSBRI visiting scientists and post-doctoral students shall be given a high priority consideration for support of the JSC intramural efforts. It is to be noted that NASA will make every effort to support intramural research through JSC post-doctoral students or visiting scientists.
- The NSBRI shall have the option of utilizing any research/academic position to fulfill this responsibility, and will not be required to provide these positions if NASA does not provide resources associated with approved JSC intramural research.

7.4 Definition of a Common Basis for NASA/NSBRI Product Identification

One of the major challenges of the NASA/NSBRI partnership will be to foster autonomy between NASA and the NSBRI so that each does not have to be bound by the inherent limitations of the other's organization, while at the same time providing for a common ground of discussion to ensure that unnecessary overlaps or cross-purpose approaches have not developed. NASA/JSC has taken on the lead implementation center management role in space biomedical research, and as part of this responsibility has developed some formalized procedures for planning, budgeting, tracking, and reporting. NASA, however, will not directly impose upon the NSBRI policies and procedures (with the exception of mandatory financial reporting) that it has in place for this responsibility.

In reviewing these processes NASA and NSBRI have agreed that a suitable concept (based on the NASA definitions) will be to utilize "deliverables" as a common point of communication. Deliverables in the NASA definition include: (1) products, data, and knowledge; (2) facilities; and (3) capabilities. These deliverables all respond to requirements for "humans in space" activities. NASA will develop and maintain lists of deliverables that it believes are important to satisfy its Code M customers. The NSBRI will be provided this list for comment/critique; further, they are invited to participate in its development and evolution to the extent they desire. This NASA deliverable list shall not be construed as scientific or technical direction from NASA to the NSBRI. However, through this process NASA and NSBRI will develop a common understanding of how each organization's activities will contribute to implementation of the overall NASA HEDS Enterprise and NASA's Strategic Plan. NASA and NSBRI will also share their management and programmatic approaches and may choose to share additional common activities when thought to be in the best interests of the CA/CAMP.

7.5 Support for NASA Program Planning

NASA engages in program planning activities to develop overall research strategies and tactical resource allocations against those strategies. While NASA Headquarters is fully responsible for this activity, NASA/JSC in its role as lead for the human elements of space flight, will provide analyses and other support activities to Headquarters in order that program formulation, tactical resource allocation, and implementation are integrated and coordinated. Since the NSBRI has a major mission responsibility that parallels this from a perspective that represents the scientific community and not necessarily NASA programmatic, NASA and NSBRI agree to the following process:

- On a mutually agreed upon schedule, the NASA/JSC Director of Space & Life Sciences and the NSBRI Director will conduct meetings or teleconference discussions that address (as appropriate but not limited to): (1) current program content; (2) implementation issues; (3) appropriate responses to advisory committee

recommendations; (4) upcoming major milestones and events; and (5) future program thrusts and direction. The Director of Space & Life Sciences will coordinate and distribute the agenda for this activity. Actions resulting from these discussions should be documented, concurred upon and distributed among the parties.

- The Director of Space & Life Sciences will coordinate, and seek advice/decisions, as required, from NASA Headquarters on issues that relate to overall program content and balance.

7.6 Addition of Work Elements

It is expected that additional specific work elements may be jointly agreed to be within the intent and overall scope of the NASA CAN and the resulting CA/CAMP. NASA and NSBRI agree that either entity can propose specific work element/projects that are within the overall scope of the NASA CAN, but by their nature would not normally be research activities included within the NSBRI core research plan. When both NASA and NSBRI agree to the implementation of a new work element/project, a project plan will be prepared and included as a supplement to the CA. Such additional work elements shall not exceed on a yearly basis 20% (individually) or 40% (total) of the total level of NASA yearly core funding. These limitations only relate to expenditure of funds within the NSBRI and do not relate to any project funding outside of NSBRI.

These limitations do not constrain core-funding levels that NASA may provide to the NSBRI to accomplish its core research; there are no limitations to the level of core funding that NASA may apply. However, a minimum level of \$30 million annually for core funding has been committed through 2007.

The specific process by which work elements are to be added is as follows:

- NASA provides a Request for Project Proposal (RPP), through the JSC Contracting Officer, to the NSBRI Director. The RPP will contain: (1) Background/Purpose/Scope; (2) Identification of deliverables/expectations; (3) Implementation guidelines (applicable policies, procedures, schedules, resources, etc.); and (4) Reference documents (if applicable). The general NASA definition of deliverables (knowledge/products, facilities, and capabilities) is applicable.
- The NSBRI will respond with a preliminary Project Proposal (submitted through the JSC Contracting Officer) that identifies the suggested approach and budget for accomplishing the project.
- NASA will review the proposed Project Plan and negotiate a final Project Plan with the NSBRI.
- If approved, the Project will be added to a list of approved projects by supplement to the CA.
- The NSBRI can also submit unsolicited Project Plans to NASA for consideration.

7.7 Review Processes

As identified in the CAN, the following review activities will be employed:

- An annual written status report (approved by the NSBRI board of directors) will be provided by NSBRI to NASA at the end of each NASA fiscal year.
- A major formal review was held following the end of the third full fiscal year of NSBRI operations. The NASA Chief Scientist will specify the content and process for future reviews. It is anticipated that reviews will be held the third year of each 5-year segment for the duration of the NSBRI CA.

7.8 Risk Analysis Approaches

NASA and NSBRI agree to the importance of attempting to understand how human health and mission/programmatic risks should be employed in space biomedical research program formulation and implementation. Therefore, NASA and NSBRI will establish a formal joint committee (membership and meeting frequency to be determined by the Director, Space and Life Sciences and the NSBRI Director) that will determine overall goals and objectives for a risk analysis effort. The NSBRI will support NASA in the actual implementation of risk analysis. This support to the risk analysis activity shall be considered part of the NSBRI core activities.

7.9 International Participation

NASA and NSBRI recognize the desirability of understanding and potentially including the efforts of NASA's international partners. In order to facilitate this interaction, the NSBRI will assign a senior individual to attend international life science research planning workshops, as required. The NSBRI will be encouraged to continue to expand its collaborations with NASA's international partners.

7.10 Education and Public Outreach Activities

NASA and the NSBRI have agreed on the importance of implementing a joint, aggressive education and public outreach effort. To facilitate an integrated and optimized approach, NASA and NSBRI will share their plans and attempt to modify, when feasible, any conflicting or inefficient activities. Education and public outreach will remain a priority objective for NASA/JSC and NSBRI and will meet the stated goals and objectives of the NASA Office of Education (Code N).

7.11 Information Technology (I/T) Utilization

NASA and the NSBRI have agreed on the importance of utilizing appropriate information technology tools to facilitate and streamline NASA/NSBRI interactions. NASA and NSBRI will share current plans and available I/T resources in order to develop both a short-term and long-term integrated I/T effort. NASA and the NSBRI agree to hold ad

hoc meetings or other activities as appropriate in order to facilitate planning and implementation in this technical area.

7.12 Collaboration in Space Medicine Efforts

The NSBRI CAN specifically stated that the NSBRI would not have direct medical care responsibilities; this remains NASA's responsibility for crew health, well being, and performance. Nevertheless, NASA has determined that NSBRI interaction with medical personnel and controlled access to medical information will benefit both organizations. Enabling the NSBRI to effectively pursue its mission and objectives requires a close collaboration between NASA operational space medicine physicians and the research/technology tasks of the NSBRI. In order to effect this collaboration, NASA will provide a medical de-brief to NSBRI management after each NASA mission. Furthermore, NASA will enable this observational participation in space medicine activities. Additionally, NASA will provide NSBRI management and discipline team leads with periodic summary briefings (updated as required) of crew health, well-being, and performance issues that are directly relevant to the mission and objectives of the NSBRI. NASA and NSBRI will agree on specific procedures by which crew confidentiality and data privacy rights will be maintained.

Similarly, the NSBRI will provide to NASA on a timely basis any knowledge or information which it believes is important to the conduct of human operations in space.

**REVIEW OF THE
NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
STRATEGIC RESEARCH PLAN**

Washington, D.C.

June 10-11, 2002

Review Committee

Chairperson: Judith Vaitukaitis, M.D.
Steve Beckwith, Ph.D.
Carolyn Huntoon, Ph.D.
Carol Scott-Conner, M.D., Ph.D.
James Snow, Jr., M.D.
Frank Sulzman, Ph.D.
Judith Tintinalli, M.D., M.S.

Consulted By Teleconference:

Allan Tobin, Ph.D.



**Office of Biological and Physical Research
National Aeronautics and Space Administration**

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EXECUTIVE SUMMARY

After an open national competition in 1997, the National Aeronautics and Space Administration (NASA) selected a consortium of seven academic institutions, led by Baylor College of Medicine, to conduct biomedical research to define adverse medical and biologic consequences associated with space travel and to identify approaches or countermeasures to prevent, minimize and reverse those adverse processes. That consortium became known as the National Space Biomedical Research Institute (NSBRI). In the first five years of its existence, the Institute has made outstanding progress.

It has:

- Developed a comprehensive research strategy that addresses key medical, physiological and technical issues associated with space travel;
- Recruited distinguished investigators from across the nation; and
- Established links to the key NASA end users, including astronauts, flight surgeons, engineers, other federal agencies, industry, and international partners.

The primary mission of the NSBRI continues to be the development of countermeasures that mitigate the health risks and consequences of space travel. The Institute plays an essential enabling role for NASA by providing unique capabilities for focused, basic and applied research that leads to development of effective countermeasures and techniques that bridge the expertise of the biomedical research community, complemented by the engineering and operational expertise of NASA. The progress of the Institute underwent independent review at its 3-year milestone and the independent committee recommended support for an additional five years. In response to weaknesses described in that report, the Institute formulated a 5-year Strategic Plan to address weaknesses cited in that initial review.

The National Aeronautics and Space Administration subsequently constituted this independent Review Committee to examine the responsiveness of NSBRI to the shortcomings cited in the 3-year review. The membership of the Review Committee appears in Attachment 1. The Review Committee's charge was to review the Strategic Plan and provide recommendations to NASA, including the soundness of the Institute's approaches to identify and resolve biomedical problems related to the health, safety and performance of astronauts during and after space flight. These issues have been elaborated in the recent Institute of Medicine report "Safe Passage", 2001 and The National Research Council report, "A Strategy for Research in Space Biology and Medicine in the New Century", 1998.

The Institute is entering an important phase, now that its infrastructure and research agenda have been established. With current and planned long-duration space flights on the International Space Station (ISS), NASA has an increasing need for the capabilities of the NSBRI, especially in the context of loss of NASA technical personnel over the years. The benefits of the NSBRI Program to NASA and to health care on earth are potentially very significant. NASA must therefore commit to adequate and stable funding for the Institute to be able to develop effective countermeasures to enhance safety of prolonged and recurrent space travel.

During its deliberations, the committee reviewed the NSBRI Strategic Plan (May, 2002) in the context of the November 2000 External Review. The committee received presentations by Dr. John Rummel, Dr. Ronald White, Dr. Bobby Alford, and Dr. Jeffrey Sutton. The committee has provided critiques and recommendations in the following eleven topics: Organizational Structure, NSBRI Teams, Research Management, Research Priorities, Radiation Health Research, Bioinformatics, Peer Review, Relationships with Complementary Programs, November 2000 External Review, President's Management Agenda, and Budget.

Topic 1: Organizational Structure

Critique:

The organizational structure of NSBRI requires clarification. The Committee could not clearly identify the lines of authority and responsibilities for senior management. Furthermore, the Strategic Plan does not adequately describe how programmatic authorities and responsibilities are distributed between NSBRI and NASA as well as how research priorities are set by those organizations.

The NSBRI advisory system appears to be functioning well in that it provides effective programmatic oversight and advice.

The NASA External Review for teams and the 5-year review for the Institute are so intertwined that the 5-year review cannot be accomplished without a concurrent review of the research conducted by team members.

Recommendations:

To optimize the research goals of NSBRI, the Institute needs to define and perhaps further strengthen its management structure and clarify the roles, responsibilities and duration of appointments for the senior management.

The Committee recommends that the NASA Chief Scientist's Review of the Institute be preceded by the review of the individual teams by no more than 2 to

4 months. This approach will streamline the review process and decrease the burden of these periodic reviews, distributed over several years. The Review Committee suggests that this recommendation supercede the recommendation of the 2000 NASA Chief Scientist's Review.

Topic 2: NSBRI Teams

Critique:

The Strategic Plan describes the two basic criteria -- scientific merit and research relevance to the NSBRI mission -- on which applications are selected. Team leaders may recommend funding of those applications relevant to the scientific thrust of their team to top NSBRI management.

The Strategic Plan does not clarify that research team members are geographically dispersed, remain at their own institutions, and are not grouped at one site. In essence they form "virtual teams." Investigators are invited to become members of a team relevant to their research proposal after their grant application is selected by NSBRI for funding.

The selection of the team leaders requires amplification. Further, the role and effectiveness of the team leaders is unclear as well as their "value added" or impact on leading the research teams. Finally, it is not clear how and whether the team leaders can ensure that projects within each team relate to each other as well as other NASA research.

Recommendations:

To optimize the team approach, NSBRI needs to pay closer attention to team management and the selection of the team leaders. NSBRI should develop a plan defining the selection, training and function of team leaders, their term of office, and their roles and responsibilities. NSBRI should consider the inclusion of NASA technical experts as potential team members. This plan should be developed in the next 6 months. Separately, NSBRI may consider evaluation of their research team approach through an independent third party to define best practices and optimize the current team approach or modify the current structure. Further, NSBRI should construct a set of simple metrics allowing them to compare team performance over time and across disciplines. (See Management of Research section below.)

These metrics may be used to determine the best allocation of resources among the teams to further the goals of the Strategic Plan.

Topic 3: Management of Research

The NSBRI plays a unique role in the development of countermeasures by supporting research that is more applied than that solicited through the NASA Research Announcements.

Critique:

At this early stage of the evolution of the NSBRI research program, it is appropriate that much of the research addresses lower Countermeasure Readiness Levels (CRL).

Recommendations:

The NASA and the NSBRI should develop metrics to track the development of countermeasures. The following metrics are suggested:

- What is the distribution of tasks by CRL or TRL (Technology Readiness Level)?
- What is the distribution of tasks and funding by criticality (likelihood & consequences)?
- How many tasks are being flown to test countermeasures or develop baseline data?

Over the next 5 years, the research program should move aggressively toward a much larger proportion of higher CRLs.

Topic 4: Research Priorities

The Strategic Plan states that the primary goal of the NSBRI is the Countermeasure Research Program. Secondary goals are education, training and outreach, along with cooperative research and development.

Critique:

The Committee strongly agrees that the top priority of the Institute should continue to be Countermeasures Research. The development of countermeasures for the health and safety of the astronaut is paramount. Health risks associated with increased flight duration may not have been adequately defined. In that setting, additional countermeasures for longer duration missions undoubtedly will need to be developed. The level of NASA's financial support for the Institute's research program is insufficient to support the level of research required to define risks and their countermeasures.

Recommendations:

The Committee recommends that NSBRI continue to maintain Countermeasures Research as its primary research focus.

At this time, efforts in education should emphasize graduate and post-graduate support in research environments in NSBRI consortia member laboratories. As the Institute and its research programs mature, educational efforts may expand to K-12. Cooperative Research and Development, Education, Training, and Outreach should be secondary to the research focus. Some elements of Cooperative Research and Development may be incorporated into the Countermeasures Research.

Topic 5: Radiation Health Research

NASA and the November 2000 External Review Report have emphasized the importance of radiation effects for humans on long duration space flights.

Critique:

NASA has developed a strategic plan for radiation health and the Johnson Space Center, as the lead Center, has developed an implementation plan. These plans take into account the NASA's distributed nature of activities and programs relevant to radiation health such as materials science, solar space physics, astrophysics, space operations, etc. Further, NASA has committed to developing and operating a beam line at the Department of Energy's Brookhaven National Laboratory (BNL), which will be used to simulate unique aspects of the space radiation environment. The NSBRI has a modest program in radiation effects, but its relation to the broader NASA program should be further refined. Since the BNL is one of the member institutions of the NSBRI, there is a good opportunity for the Institute to play a key role in the NASA efforts.

Recommendations:

NASA and the NSBRI should clarify the role that the radiation effects team should play in the overall program to reduce health risks associated with exposure to space radiation.

NSBRI should consider the addition of NASA JSC technical experts on radiation to the radiation effects team.

Topic 6: Bioinformatics

Bioinformatics is essential for research in the 21st century. The nature of research is evolving rapidly and commonly requires high throughput technologies that generate vast volumes of data that require special bioinformatics capabilities for rapid analysis. The current plan fails to include a comprehensive bioinformatics plan. Bioinformatics is an essential infrastructure component required by all research teams.

Critique:

Important previous space flight information and other research data exist in NSBRI, JSC and with individual investigators. Bioinformatics is a crosscutting discipline with sets of analysis tools that span all the research of the NSBRI. The Strategic Plan does not include a functional approach.

Recommendations:

Bioinformatics should be a prominent part of NSBRI's Strategic Plan. Tools should be developed to model and visualize data and to assess whether unexpected relationships may be present in existing databases for factors not yet identified. A comprehensive approach needs to be developed. Both NASA and the Applied Physics Laboratory at Johns Hopkins, a member of the consortium, hold world-class expertise in this area that should be tapped. The Strategic Plan should be modified to include bioinformatics as a key crosscutting set of tools essential for modern research.

Topics 7: Peer Review

Critique:

The recently modified Peer Review process is fair and objective.

Recommendations:

Continue to maintain the high quality and objectivity of the Peer Review process.

Topic 8: Relationships with Complementary Programs

Critique:

There are several programs in the nation that are complementary to the NSBRI. Many Federal agencies support preclinical research into the mechanisms responsible for microgravity-associated risks and other space flight effects. For example, the National Institutes of Health is the premiere funding agency for the study of bone and muscle abnormalities and various other disease conditions. Development of many procedures and technologies for space flight applications can be adapted from such complementary research programs for effective therapies to restore or promote health on Earth.

Recommendations:

Leverage NASA's investment in space travel related health risk research in collaboration with related federal research programs. Whenever possible, develop a collaboration with federal agencies on research, for example, in areas such as bone, muscle, metabolism, and health monitoring technologies. One

would expect that many technologies and countermeasures developed to minimize or prevent the consequences of space travel can be adapted to have practical value in health care delivery, just as electronic developments in the formative NASA years resulted in several technologic spinoffs to society's benefit.

Topic 9: November 2000 External Review

Critique:

The Review Committee concludes that NSBRI has effectively addressed the major concerns raised in the first NASA Chief Scientist's Review.

Topic 10: President's Management Agenda

The overall goals, objectives, and approaches are being utilized by NASA and the NSBRI as related to the President's Management Agenda.

Critique:

The establishment of the NSBRI is entirely consistent with the President's Management Agenda in terms of strategic management of human capital, competitive outsourcing, improved financial performance, expanded electronic government, and budget performance and integration.

However, without stable and appropriate funding from NASA, the NSBRI will not realize its potential and satisfy the President's Management Agenda.

Recommendations:

NASA should provide stable and sufficient funding for the NSBRI (See Budget section below).

Topic 11: Budget

Critique:

The overall NSBRI budget is inadequate for the scope of research needed to assure the *safety and health* of astronauts, especially in view of the unknown risks of future long-term space travel and travel into deeper space. NSBRI is operating in an environment in which the projected level of research funding is uncertain and varies sharply from year to year. It is essential that a grant-awarding entity for programmatic research have reasonable stability of financial support. Instability will lead to expert investigators moving to other research and will possibly place future astronauts at unacceptable risks for prolonged and deep space travel.

A chaotic situation that exists because the proposed reduction in funding for FY 2003 is severe and instability in the funding process disrupts the continuum of research, makes it impossible to achieve the goals of Countermeasure Research. The funding decrease threatens the pool of investigators leading this field. Furthermore, the baseline budget in the Strategic Plan is inadequate to meet the goals of the Strategic Plan. Even the full program budget fails to meet the needs in view of the proposed increased duration and repeated space flights.

The complexity and implications of NSBRI research programs are comparable to clinical research supported by the NIH, but the average allocation per project at NIH is significantly greater than that for the NSBRI-funded projects. The original budgetary discussions of an annual NSBRI budget of 50 million to 100 million dollars, in addition to the baseline Biomedical Research Program, would be appropriate to support research into the health risks associated with space flight. The need is urgent because health risks are likely to increase cumulatively with each subsequent International Space Station mission. Furthermore, data that can be collected and analyzed now will provide the substrate for future refinements of the health care system in space, will improve crew selection and rehabilitation, will improve health care maintenance in space, and will affect environmental and engineering adaptations for future long-range space missions.

Recommendations:

NASA should increase and stabilize the NSBRI budget. This is essential to the success of the program and the development of the next generation of researchers. Although NSBRI funding restoration and increase is recommended, it should not be at the cost of NASA's Biomedical Research and Countermeasures Program. Without adequate funding, many talented investigators will abandon this research area and promising researchers will pursue more attractive research areas. The foregoing will preclude future safe space travel.

The Institute should consider the use of NIH-style center support mechanisms to provide an appropriate funding mechanism for more complex research queries that require a research team with complementary expertise required to develop novel research tools and technologies required by the team. A new NASA investment in 2-3 comprehensive centers of this type can lead to an integrative approach into developing countermeasures that are complex and essential and unlikely to be effectively addressed by the classic individualized R01 approach. This approach may include NASA engineers, physicists, and computer programmers working side-by-side with NSBRI investigators. The R01 mechanism remains essential for research that is hypothesis-driven and not heavily dependent on advanced technologies.



Attachment 1

**NSBRI Strategic Research Plan Review Meeting
Universities Space Research Association
(Washington Design Center)
300 D St. S.W., Suite 801
June 10-11, 2002**

Review Committee

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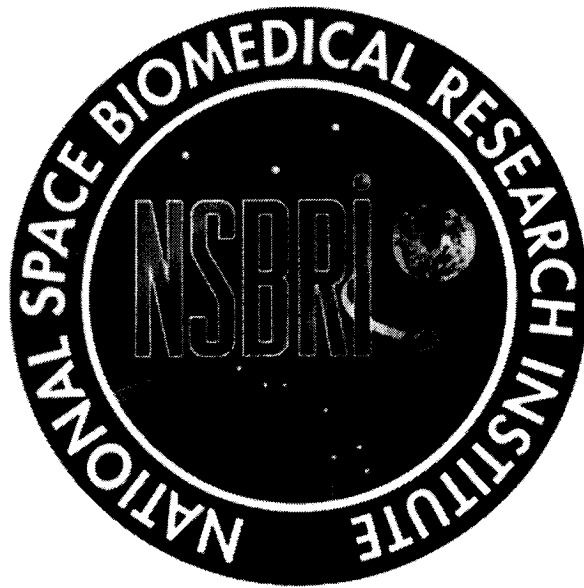
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Appendix C

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



***CORE RESEARCH PROGRAM
YEAR 6 - FY 2003***

September 30, 2003

**National Space Biomedical Research Institute
Core Research Program – Year 6
FY 2003
September 30, 2003**

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**NSBRI RESEARCH PROGRAM
BONE LOSS**

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Bloomfield, S. A.	PI	Texas A&M	Bone and Muscle Recovery from Simulated Microgravity 4
Hogan, H. A.	CO-I	Texas A&M	
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Baldwin, K. M.	CO-I	UC, Irvine		
Ruff, C. B.	CO-I	Hopkins/SOM		
Beck, T. J.	CO-I	Hopkins/SOM		
Oden, Z. M.	CO-I	UT-Houston		
Potember, R. S.	CO-I	Hopkins/APL		
Burman, K. D.	CO-I	Wash. Hosp. Ctr.		
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Antich, P. P.	CO-I	UT-SW		
Pak, C. Y. C.	CO-I	UT-SW		

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Susan A. Bloomfield, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT:	Bone and Muscle Recovery from Simulated Microgravity

Project Executive Summary

These investigations are designed to determine how mechanical interactions between bone and skeletal muscle contribute to the recovery of bone mass and bone strength after exposure to prolonged microgravity. Given evidence for the risk of bony injury when large muscle contractile forces are imposed on weakened bone, these are key issues to solve to minimize risk of injury to human astronauts during and following exploration class missions. The consequences of this disrupted muscle-bone balance for the recovery of bone after skeletal unloading are not well understood. Hence our first objective was to define a time course for recovery of both skeletal muscle and bone functional properties after 28 days of hindlimb unloading (HU) in an adult rat model. The data clearly indicate that bone mineral density (BMD) at the proximal tibia, a skeletal site sensitive to changes in mechanical loading, decreases about 5 percent over 28 days of HU, and declines even further during the next 28 days of normal ambulation (to about 92 percent of baseline values). On the other hand, strength of the ankle plantar-flexor muscles declines more rapidly during HU but then by 14 days of recovery has recovered to 98 percent of baseline values. Force-frequency relationships are also fully normalized by 14 days of recovery. Hence there is a clear functional mismatch between these two tissues between 14 and 28 days of recovery, with BMD (a surrogate measure of bone strength) still declining and muscle function recovered to 110 percent of baseline values by 28 days of cage activity. Other outcome measures should help define tissue level mechanisms promoting or retarding recovery of bone following unloading, including the role of bone marrow osteoprogenitor cells. Thus far, our cell culture data do not present a clear picture of the functionality of these precursor cells; more work remains to be done here. Histomorphometry studies of mid-tibia cortical bone reveal that bone formation rate at the periosteal surface is nearly shut down after 28 days of HU, but recovers nicely after 28 days of normal ambulation. Similar studies of proximal tibia cancellous bone now in progress should reveal in year three if there is any recovery of normal bone formation activity in this bone compartment that usually responds more rapidly to changes in loading.

We have begun studies on the effectiveness of several mechanical loading regimens and two pharmacological agents in promoting bone recovery and minimizing the functional mismatch between bone and muscle after 14 days of recovery. Preliminary results indicate that parathyroid hormone (PTH, a formulation of which is now FDA-approved for clinical use in humans) is remarkably effective in promoting recovery of proximal tibia BMD during recovery from 28 days of HU. Important groundwork is described in this progress report towards testing a mechanical loading intervention during recovery which utilizes stimulated muscle contractions, in an attempt to answer whether intensive weight training (in a human returned from spaceflight) can safely increase BMD while also hastening muscle recovery.

Our collaborative group has published two articles in the past year resulting directly from this funding, with another manuscript in preparation. Research presentations on these data were made at the American Society for Bone and Mineral Research (9/03), the Bioastronautics meetings (1/03) and Experimental Biology meetings (4/03).

Interim results and budget cuts during year two delayed progress in testing some of the interventions and will likely result in dropping the aerobic exercise intervention during recovery during year three. However, since most available data on rodents and humans indicate that resistance-type training is usually superior for minimizing bone loss with disuse, we feel this is a minor disadvantage.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Mark E. Bolander, M.D.
ORGANIZATION:	Mayo Clinic Rochester - Rochester
PROJECT TITLE:	Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure

Project Executive Summary

The NSBRI Conference that was convened in Clear Lake, Texas, on November 16-17, 2000, identified fracture healing during space flight as an area where further information would be required to appropriately prepare for long-term space missions, and developing countermeasures to restore normal fracture healing was identified as a priority for current research. The RFA dated February 22, 2000, (NSBRI II 00-01) requested studies evaluating the effect of space flight on fracture healing and developing countermeasures. This application is submitted in response to that RFA.

Our current understanding of bone physiology suggests that fracture healing will be abnormal in the microgravity environment. This hypothesis is supported by two published studies, the first an abstract reporting abnormal healing in rats undergoing hindlimb unloading, the second a manuscript (in Russian) that describes abnormal fracture healing in five rats with fibula fractures flown on Cosmos-2044. This latter study reports that abnormalities seen in fracture healing after space flight were duplicated in the hindlimb-unloading model.

The goals of the experiments proposed in this application are 1) to confirm the previous reports that microgravity adversely affects fracture healing, and 2) to determine if ultrasound treatment, which has been shown to accelerate fracture healing in clinical studies, will reverse the impaired cellular events in fracture healing that are related to microgravity. If ultrasound does not act as an effective countermeasure we will undertake detailed evaluation of our histologic samples to identify potential targets for other countermeasures.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Carlos M. Isles, M.D.
ORGANIZATION:	MCG Research Institute, Inc.
PROJECT TITLE:	Therapeutic Modulation of Systemic Glucose-Dependent Insulinotropic Peptide Levels to Counteract Microgravity-induced Bone Loss

Project Executive Summary

Our long-term goal is to understand the molecular mechanisms of bone formation, maintenance and repair. Weight-bearing is essential for bone formation and maintenance. In the absence of load, for example, in the microgravity environment of space, bone tends to atrophy. We hypothesize that the detrimental effect of diminished gravitational load can be overcome by exploiting the hormonal cues received by bone. Our primary candidate for effecting this control is the enteric hormone, glucose-dependent insulinotropic peptide (GIP), secreted from the small intestine in direct response to nutrient intake. The accepted function of GIP is coupling insulin secretion to blood glucose elevations. However, our group was the first to demonstrate an additional function for GIP, namely the stimulation of bone formation(1-3). Functional GIP receptors are present on bone-forming cells *in vivo* and activation of these GIP receptors leads to an increase in bone formation and, conversely, an inhibition of bone resorption.

As part of our NSBRI funded project we have performed experiments exploring different levels of hormonal control of bone formation as potential sites of GIP action. We have found important new controls of bone formation both produced systemically (endocrine control) and locally (paracrine control). Findings to report for this annual report include: (1) new findings on the molecular controls of bone formation (2) new findings on how nutrients we ingest can be directed to the bones to be utilized for bone formation and (3) the discovery of a novel hormonal loop in bone where the bone resorbing cells, osteoclasts, regulate the bone forming cells, osteoblasts, through local production of bone active hormones.

(1) Molecular controls of bone formation: Our work in this area has focused on two factors *Tbx2* and ERV-9 LTR. *Tbx2* belongs to a family of developmental transcription regulatory factors. We evaluated whether the gap junction protein Connexin43 (Cx43), an important regulator of osteoblast function and bone development, may be a downstream target gene regulated by *Tbx2*. Our findings indicate that the promoter of Cx43 is repressible by *Tbx2*, both in cultured osteoblast-like cells *in vitro* and also likely in the developing embryo. We have found that parathyroid hormone can modify *Tbx2* expression and are now evaluating whether it is also modified by GIP.

The long terminal repeats (LTRs) of the human endogenous retrovirus ERV-9 is different from other endogenous retroviruses in that they have recurrent motifs capable of binding to cognate transcription factors. We have found that embryonic bone stem cells (but not mature bone cells) contain these ERV-9 LTRs suggesting a role in bone cell development. We have begun the characterization of bone stem cells and plan to define the role of environmental factors on bone stem cell development.

(2) Nutrient utilization for bone formation: Our work in this area has focused on GIP as an integrative hormone with effects on both the vasculature (to maximize postprandial nutrient

absorption) and effects on the bone to stimulate new bone formation. We propose that by therapeutically elevating GIP levels, coupled with strict dietary control, it will be possible to mitigate the impact of microgravity.

It is well known that after a meal the blood flow to our hepatic artery decreases and the portal vein circulation increases, the purpose of this alteration in blood flow is to increase the absorption of nutrients from the recently ingested meal. These changes can be reproduced by infusion of GIP, suggesting that GIP is the hormone responsible for these changes in blood flow after a meal. In order to determine how GIP could have apparently opposing effects on blood flow in these different vascular beds we isolated primary hepatic artery and portal vein endothelial cells and found that GIP's action differed between these endothelial cells. In portal vein, but not hepatic artery endothelial cells, GIP increased production of the potent vasodilator nitric oxide. In contrast, in hepatic artery, but not portal vein endothelial cells, GIP increased release of the potent vasoconstrictor endothelin-1. These findings highlight the importance of GIP as a hormone involved in nutrient utilization.

GIP is also an important hormonal link between nutrient ingestion and bone formation. To define GIP's role in normal bone formation we have generated transgenic mice with GIP levels up to ten times normal. We have examined the bone phenotype in these GIP-overexpressing transgenic mice and find that they have a significantly increased bone mass and the bones of these animals, assessed by biomechanical testing, are stronger than those of control mice. We are currently performing tail suspension experiments in these animals. Our hypothesis predicts that these mice will be protected against bone loss in the simulated microgravity environment.

Experiments to be performed over the coming year should give us a much better understanding of GIP's effects on bone. We have obtained GIP receptor knockout mice from Dr. Yamada in Japan and as predicted these mice have about a 13 percent lower bone mass than normal control mice. We are currently performing tail suspension experiments on these mice, we would predict that these mice, having lost the protective effect of GIP on bone formation, would be much more prone to lose bone under simulated microgravity.

(3) Paracrine control of bone turnover: Our work in this area has focused on two hormones, PACAP and ACTH.

PACAP (Pituitary Adenylate Cyclase Activating Peptide) is known to regulate proliferation, differentiation, and apoptosis in some cell populations. In addition, PACAP regulates metabolism and the cardiovascular, endocrine, and immune systems although, specific PACAP actions on many tissues is still poorly defined. The PACAP receptor belongs to the seven transmembrane G-protein coupled family of receptors that also includes GIP, PTH/PTHrP, calcitonin, secretin, VIP and GLP-1. We have found data specific high affinity PACAP receptors in osteoblastic-like cells and our data suggests that PACAP may play a role in osteoblastic proliferation and differentiation. Thus, our data suggests another mechanism for nutrient regulation of bone formation, through PACAP release from nerves innervating the bone, activated upon nutrient ingestion.

Finally, we have done experiments examining a role for ACTH in bone formation. Another nutrient related hormone which has been reported to have bone specific effects is leptin. Leptin ultimately acts through the melanocortin receptors. Melanocortin receptors belong to the seven transmembrane domain, G-protein coupled family of receptors. Five melanocortin receptors have been described which are widely expressed in the body, including skin, brain, adrenal

glands, adipocytes, gut and other peripheral tissues. These receptors are activated by fragments derived from a larger molecule, pro-opiomelanocortin (POMC) and include: ACTH, α β and γ -MSH, and β endorphin. Traditional roles for these peptide hormones include regulation of cortisol secretion, skin pigmentation and pain perception. Although the pituitary gland is the traditional site for synthesis, secretion and processing of POMC-derived fragments, it has become clear that other tissues can also synthesize and secrete POMC fragments including keratinocytes, melanocytes, endothelial cells and immune cells among others. Among the latter both lymphocytes and macrophages have been reported to express and secrete POMC fragments. Since osteoclasts in bone are derived from macrophages we investigated whether POMC and the melanocortin receptors were expressed in bone and bone cells. We found that the five known melanocortin receptors are expressed in normal rat bone as assessed by *in situ* hybridization. These receptors were variably expressed in different osteoblastic-like cell lines with the MG63 cell line expressing four out of five melanocortin receptors. In attempt to determine the source of ligand for these melanocortin receptors, normal rat osteoclasts were probed for the POMC gene by Northern blot. Osteoclasts did in fact express the POMC gene although at a lower level than normal rat pituitary and conditioned osteoclastic tissue culture medium contained ACTH. Thus, bone cells contain the elements for a paracrine hormonal loop where osteoclasts express POMC and osteoblasts express melanocortin receptors.

Thus, we have made significant new advances in our research objectives this year and have gained new insights into the controls involved in bone formation. During the next calendar year we propose to extend this year's findings and thus, increase the countermeasure readiness level of GIP as a treatment for microgravity-induced bone loss.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Gerard Karsenty, M.D., Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Leptin as a Regulator of Bone Formation in Microgravity

Project Executive Summary

Original Aims

To determine whether leptin controls bone mass by releasing a humoral substance following its binding to its hypothalamic receptor.

To determine whether the sympathetic nervous system is involved in mediating leptin control of bone formation.

To determine whether a naturally occurring soluble form of the leptin receptor can prevent leptin inhibitory action on bone formation.

Key Findings

Several key findings were made during the previous year of funding. These can be summarized as follows:

Leptin uses different pathways to control bone mass and body weight.

The concentration of leptin in blood is not a good indicator of its action on bone formation.

Neurons present in the hypothalamus and controlling bone formation have been identified.

The mediator coming out of these neurons and affecting bone formation is not present in blood.

Circulating leptin level controls bone mass.

Impact of Findings

These findings confirmed largely our working hypothesis that there is a brain-derived neuronal control of bone mass. They lead us to propose new experiments to identify the mediator relaying information from the brain to the bone cells.

In the coming year we intend to use mutant mouse strains deficient in various neuromediators to identify the mediator of leptin action on bone mass. Once we will have identified this mediator we will generate an inhibitor of this mediation and we will use it in ovariectomized animals to determine whether it can be used to prevent the development of osteoporosis.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Clinton T. Rubin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	A Biomechanical Countermeasure for Disuse Osteopenia

Project Executive Summary

Original Aims of the Project

Osteoporosis, the progressive loss of bone density and strength which cripples tens of millions on our planet, distinguishes itself as perhaps the greatest physiologic obstacle to an extended human presence in space. The principal objectives of this proposal are to establish the efficacy of a unique, biomechanical countermeasure to inhibit bone loss in an animal model of disuse osteoporosis, and correlate this regulatory influence to the expression patterns of several genes critical to bone formation and resorption. Using a ground based model of microgravity, the tail-suspended rat, we have shown that brief exposure (10 minutes) to extremely low magnitude (0.25g, engendering < 5 microstrain), high frequency (30-90 Hz) mechanical signals will inhibit the bone loss which typically parallels disuse, even though 10 minutes of full weightbearing failed to curb this loss. Longer-term experiments in sheep have shown this stimulus to be strongly anabolic, increasing bone mineral density, trabecular number and connectivity, and improving bone strength.

In a series of four specific aims, we are using several morphometric assays on the mouse model of tail-suspension to rigorously establish the efficacy of a specific mechanical signal (10 minutes at 30 Hz, 0.3g; parameters being used in clinical trials to inhibit bone loss in the elderly) to inhibit and/or reverse 28 days of disuse osteopenia. In an effort to understand the mechanisms by which this signal is anabolic, we will also monitor the temporal and spatial expression of nine genes, each indicative of a specific process of bone formation or resorption. The use of the mouse is facilitating many aspects of the protocol, including comprehensive genomic profiling and expedited access to spaceflight. Considering that many flight opportunities are brief and thus do not permit long term morphologic adaptations in bone to occur, combining the molecular with the tissue level strategies will facilitate establishing countermeasure efficacy even following short term exposure to microgravity. In essence, this work represents a critical step in establishing a physiologically based, non-pharmacologic, non-invasive treatment for osteoporosis, for use on earth or in space.

Key Findings

Thus far, the project has demonstrated that the low-level mechanical intervention is powerfully anabolic, and can effectively inhibit the bone loss which parallels disuse. As importantly, using three distinct strains of mice, we have determined that the response of the skeleton to disuse is strongly dependent on the genomic makeup of the animal, and that the responsiveness of the skeleton to the signals are dependent on the animal strain and the site examined.

Impact of Findings

These findings point to a unique, non-pharmacologic, non-invasive means of controlling bone loss in a microgravity environment. This biomechanical intervention may potentially displace the need for time consuming (and relatively ineffectual) exercise regimens, or replacing the need for pharmacologic countermeasures (and the potential long-term side effects that they may

cause). Importantly, promising results from preliminary clinical trials on post-menopausal women, girls with osteoporosis, and children with cerebral palsy, also indicate that this therapy may work for the 20 million people on earth who suffer from osteoporosis. This work may also contribute to identifying the genetic basis for those at greatest risk of the diseases.

Plans for the Coming Year

Studies will continue to identify the strain-specific sensitivity of the skeleton to disuse and/or mechanical stimulation, and efforts will begin to determine those genes that are involved in regulating the process. Work funded by NASA has begun, in the “definition” phase, to determine if this biomechanical intervention can be used effectively on astronauts in the ISS.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Mitchell B. Schaffler, Ph.D.
ORGANIZATION:	Mount Sinai School of Medicine
PROJECT TITLE:	Resorption Suppression and Bone Health in Disuse

Project Executive Summary

Osteoporosis in higher mammals due to loss of normal mechanical loading results from elevated osteoclastic resorption. Thus, targeting osteoclasts to prevent bone loss seems an obvious countermeasure strategy. To that end, during the last two years our NSBRI research (*Resorption Suppression and Bone Health in Long-term Disuse*), we examined whether a clinical bisphosphonate prevented bone loss in dogs subjected to long duration single limb immobilization. We are testing the hypotheses that 1) Long-term suppression of bone remodeling in disuse will successfully maintain bone mass, microarchitecture, stiffness and strength, but will result in compromised fracture resistance properties; and 2) Decreased mechanical usage in the presence of an antiresorptive agent results in loss of osteocyte integrity and accumulation of bone with impaired viability. Bone health is assessed from conservation of bone mass, microarchitecture, tissue mechanical properties and from in situ assessments of osteocyte viability.

To date we have completed the in vivo studies, as well as bone density and histomorphometric analyses. Our studies reveal some important and surprising results. In particular, we found that bisphosphonates were only partially effective in attenuating long-term bone loss resulting from long-term disuse. Risedronate treatment of dogs subjected to long duration single limb immobilization resulted in a 30 to 50 percent reduction in bone loss compared with non-treated disuse animals. While we must consider any conservation of bone in disuse osteoporosis to be beneficial, we do not yet know whether this treatment reduces bone resorption enough such that bone that remains after long-term disuse can make a complete recovery when loading is restored. Moreover, these observations stand in contrast findings for other osteoporoses where bisphosphonates more effectively inhibited bone loss. Thus, these results suggest that disuse is different from other osteoporoses in its sensitivity to anti-resorptive treatment.

In the coming year, we will complete our histomorphometric analyses of tissues. We will also undertake confocal microscopic studies of osteocyte integrity and perform the biomechanical testing needed to determine whether treated bones maintain their mechanical properties in proportion to the amount of bone conserved, and determine whether long-term bisphosphonate treatment adversely affect bone tissue mechanical properties during disuse.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Jay R. Shapiro, M.D.
ORGANIZATION:	Medstar Research Institute
PROJECT TITLE:	Defining and Preventing Bone Loss: A Microgravity Model

Project Executive Summary

Muscle atrophy and bone loss are major complications of spinal cord injury (SCI), chronic bed rest and exposure to microgravity. Space medicine research has amply documented the extent to which muscle and bone loss may impair strength and increase fracture risk. We propose that the SCI patient can serve as a surrogate for studying microgravity exposure. A primary objective of this research program is to limit the extent of bone loss in SCI patients by treating with a potent intravenous bisphosphonate, zoledronate for a period of one year. The zoledronate effects on bone will be measured using bone density values and femur scan structural analysis as the indicators of bone integrity. We will determine the effects of zoledronate on biomarkers of bone resorption and formation and on serum calcitropic hormone levels. To study the process of muscle atrophy when weightless, we will determine the relationships between changes in thigh muscle cross-sectional area measured by CT scan, muscle biopsy immunohistochemistry, muscle protein translation markers and markers for protein synthesis activation and protein degradation. To further understand mechanisms involved in bone loss we will determine sequential changes in femur bone geometry and structural parameters obtained from DEXA scans by established 2-D curved beam analysis methods. Using femur CT images we will measure changes in femur bone dimensions and will apply 3-D finite element analysis to estimate fracture risk. The new time-of-flight mass spectrometer will permit measuring the excretion of zoledronate in urine and plasma levels. We will compare these to radiologic measurements and bone biomarkers. The objectives of this research are: 1) to develop a regimen for minimizing bone loss in SCI subjects that may be appropriate for astronauts during extended microgravity exposure, and 2) to investigate mechanisms related to muscle and bone loss during weightlessness, and 3) to explore the SCI patient as a surrogate for the investigation of microgravity induced musculoskeletal atrophy.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Carolyn L. Smith, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Receptor Countermeasures to Bone Loss in Microgravity

Project Executive Summary

The prevention of bone loss due to skeletal unloading is a complex problem and the reasons for this loss have not been elucidated. The overall goal of the bone team of NSBRI is to develop countermeasures that will not only prevent quantitative loss of bone, but also maintain bone strength. Measures that simply prevent resorption may maintain mass, but may block the necessary remodeling that ensures adequate bone strength. Studies to date suggest that good nutrition and exercise regimes will be insufficient to achieve this goal so pharmacological alternatives must be considered. The biological actions mediated by the estrogen receptor (ER) and vitamin D receptor (VDR) play key roles in the normal control of bone growth and skeletal turnover that are necessary for skeletal health. These receptors act by controlling the differentiation and/or function of osteoblasts and osteoclasts, and other cell types within the bone and bone marrow microenvironment as well as playing a role in calcium absorption (VDR). *We hypothesize that the appropriate combination of an agent that will improve calcium absorption and encourage bone formation (VDR agonist) and an agent that will reduce bone resorption (selective estrogen receptor modulators [SERM]) will achieve the goal of maintaining bone mass and bone strength.* To test this we have initiated studies to:

1. Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to modulate osteoblastogenesis, mature osteoblast function and osteoclastogenesis *in vitro* and *in vivo* and
2. Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to prevent bone loss in the hindlimb suspension model of skeletal unloading.

Effects of unloading and the countermeasures will be assessed by: a. Measuring changes in bone mineral density, histomorphometry, mechanical strength testing and biochemical markers of bone metabolism, b. Determining the effects of these treatments on osteoblastogenesis and osteoclastogenesis and function, and c. Characterizing gene expression profiles in bone resulting from skeletal unloading and administration of the countermeasures.

Our results to date indicate that ligands of both the ER and VDR possess the ability to attenuate bone loss in the rat hindlimb suspension model of skeletal unloading. Collectively, these studies will lead to a better understanding of the changes associated with skeletal unloading and will test the utility of VDR agonists and SERMS as countermeasures.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Joseph E. Zerwekh, Ph.D.
ORGANIZATION:	UT Southwestern Medical Center at Dallas
PROJECT TITLE:	Prevention of Microgravity-Induced Stone Risk by KMgCitrate

Project Executive Summary

The formation of a renal stone during space flight may have serious negative effects on the health of the crewmembers and the success of the mission. Ground-based studies, as well as a limited number of space flight studies, have clearly demonstrated an increased risk for kidney stone formation as determined from the composition of the urinary environment. Increased bone resorption raises urinary calcium and the urinary state of saturation with respect to the calcium salts, calcium oxalate and brushite. However, documented changes in other urinary components such as citrate, pH, and magnesium appear to also raise the risk for the formation of not only calcium oxalate and calcium phosphate stones but also uric acid stones as well. Nutritional modifications to counter the tendency toward stone formation might include increased fluid consumption and supplementation with an appropriate nutraceutical that would decrease the risk of stone formation by increasing urinary pH and inhibitor concentrations. The hypothesis to be tested in this project is that potassium magnesium citrate supplementation will attenuate the increased risk for stone formation and diminish microgravity-induced bone loss. This hypothesis will be tested during five weeks of bed rest in normal volunteers through three specific aims: 1) assess the efficacy of supplementation with potassium magnesium citrate (KMgCit) in preventing microgravity-induced increased risk of renal stone formation; 2) assess the efficacy of supplementation with KMgCit in reducing microgravity-induced increases in bone resorption and urinary calcium losses; and 3) evaluate the effect of KMgCit supplementation in averting diminished muscle magnesium and potassium concentrations that may occur during microgravity-induced muscle atrophy.

This study is being conducted as a randomized, double-blind, placebo-controlled trial. As such, it is not currently possible to evaluate the effectiveness of this therapy in reducing stone risk and skeletal bone loss until study completion. Although an interim analysis could be performed, doing so could affect the overall statistical power of the study and necessitate inclusion of additional subjects. Despite this limitation, several key observations have continued to be evident in the second year of this study. For the entire group of subjects, mean urinary pH increased during bedrest, most likely reflective of the alkalizing effect of KMgCitrate. This notion is also supported by increases in urinary potassium, magnesium, and citrate for the group. Net gastrointestinal absorption of alkali also increased significantly for the group. Overall, these changes resulted in no significant change in urine saturation with respect to the stone forming salts of calcium oxalate or sodium urate. In addition, the saturation of urine with respect to undissociated uric acid did not show any significant change from baseline. It is important to note that the lack of change in urinary calcium oxalate saturation was evident despite a marked and significant increase in urinary calcium concentration during bedrest. This rise in urinary calcium concentration and, to a lesser extent in urinary phosphate excretion, is most likely the result of increased bone resorption during skeletal unloading. This is supported by the significant increase in urinary deoxyypyridinoline excretion during bedrest, a marker of increased bone resorption. This response is further supported by the fall in serum parathyroid hormone. At present, it is not possible to discern whether KMgCitrate treatment may have attenuated the apparent increase in

bone resorption. This will only be able to be addressed upon completion of the study and comparisons between the placebo and KMgCitrated-treated groups performed.

The current findings for the entire group of studied subjects does not deter from the original hypotheses of this study. The group data are in part consistent with the original hypothesis that provision of alkali, as KMgCitrated, would attenuate the increased stone risk associated with spaceflight and its Earth-based counterpart, bed rest. The observed lack of change in urine saturation with respect to calcium oxalate, despite significant increases in urinary calcium during bed rest, supports this notion. It is not yet clear what effect this agent may have on microgravity-induced bone loss since we did observe changes consistent with increased bone loss. Ultimate proof that KMgCitrated attenuated increased bone resorption during bed rest will require a comparative analysis between placebo- and KMgCitrated-treated subjects upon study completion. Finally, we have not yet analyzed muscle biopsies for magnesium and potassium concentrations. We will perform this analysis upon study completion but before the code is broken for the study. This aspect of the study is to determine if KMgCitrated supplementation can prevent atrophy-related loss of muscle magnesium and potassium.

There are no anticipated changes in the research plan for the coming year. We hope to complete the last six subjects to be studied in the first half of the year and to complete all laboratory analyses and data assessment by early summer 2004.

**NSBRI RESEARCH PROGRAM
CARDIOVASCULAR ALTERATIONS**

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RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Mohamed A. Bayorh, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model

Project Executive Summary

Exposure to microgravity or simulated microgravity in humans causes cardiovascular deconditioning with orthostatic hypotension and tachycardia. Post-flight orthostatic intolerance is a dramatic physiologic consequence of human adaptation to microgravity made inappropriate by a sudden return to normal gravity. Loss of appropriate cardiovascular reflexes contributes to the cardiovascular deconditioning, but the specific mechanisms remain uncertain. The endothelium is now recognized to play a critical role in the regulation of vascular resistance and blood pressure through the release of nitric oxide and/or prostacyclin. The objective of the proposed studies is to test the hypothesis that the post-suspension hypotension in rats following simulated microgravity involves elevated levels of prostacyclin and/or nitric oxide and, thus, can be attenuated by specific inhibitors of these vasodilatory factors. Using the 30° tail-suspended (hindlimb-unloaded) rat model, the roles of prostacyclin and nitric oxide in post-suspension hypotension are being evaluated. For the coming year we will continue to examine gender differences in the post-suspension hypotensive response.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Donald M. Bers, Ph.D.
ORGANIZATION:	Loyola University Chicago
PROJECT TITLE:	Integrative Cardiac Myocyte Model: Ion Channels, Ca and Contraction

Project Executive Summary

Specific Aims

- Develop a more up-to-date electrophysiological model of cardiac myocytes.
- Incorporate new Ca transport data on SR Ca uptake, release and Na/Ca exchange.
- Extend the model to include cooperative Ca-dependent contraction and relaxation.
- Implement model in highly accessible computational formats.

Accomplishments

Up-to-date electrophysiological model. 1. We have completed the first major version of our user-friendly computational model of cardiac ion currents and Ca regulation (LabHEART 4.7) and published a manuscript describing it in the *American Journal of Physiology* (Puglisi and Bers). We have made this model freely available for download from our website (<http://www.lumc.edu/physio/bers>). Over 500 people have downloaded the program (in 38 countries) and it has already been used successfully in teaching medical and Ph.D. students. 2. We used this model to determine the relative contributions of two key factors contributing to arrhythmogenesis in heart failure (upregulated Na/Ca exchange and downregulated inward rectifier potassium current, I_{K1}). 3. We have added versions for epi-, mid- and endocardial ventricular myocyte which helped a collaborating group (Dr. Andrew McCulloch) to incorporate our cellular model into their more integrative whole heart model.

New Ca transport version of model. We have updated LabHEART 4.7 model in several ways already. 1. We have further updated ionic currents (e.g. including important characterization and subdivision of transient outward currents, $I_{to,f}$ & $I_{to,s}$; LabHEART 4.9x). 2. We have added the facility that the user can modify the equations that describe the ionic currents and transporters (LabHEART 5.0). 3). We have also overhauled the model (Shannon-model) to include a more appropriate cellular geometry and compartments based on experimental data (including junctional cleft and subsarcolemmal compartments) and used more up-to-date experimentally tested expressions for Ca current, SR Ca release, SR Ca-ATPase and Na/Ca exchange. This major revision is currently being written up for publication and also being ported to a more versatile computational format (from that in which it was developed). 4. We have used this new model to help distinguish the relative importance of 3 factors that contribute to reducing SR Ca content in heart failure: a) reduced SR Ca-ATPase, b) increased Na/Ca exchange function and c) increased diastolic SR Ca leak, each of which we have measured experimentally. This work will be published in *Circulation Research* in October (Shannon, Pogwizd and Bers).

Extend model to include myofilament properties. 1. In parallel with the above, we have developed a novel cardiac myofilament model that includes realistic representations of the steep cooperative force- $[Ca]_i$ relationship, the length-dependence of myofilament activation and the load-dependence of contraction duration. This used local filament nearest-neighbor interactions and Monte Carlo simulations. 2. This work was written up and published as a full paper in the *Biophysical Journal* (Rice and deTombe). 3. This sort of Monte Carlo simulation is not practical

for incorporation into a cellular ion channel-Ca transport model. So, we have developed a novel ODE (ordinary differential equation) version of this model which retains reasonably well the important characteristics. This version should be practical to incorporate into our current ion channel-Ca transport model.

Highly accessible computational formats. This has been an ongoing thrust in all of the above aims. 1. LabHEART 4.7 is the prototype in user friendly version of the model for both teaching and for use by other scientists in the field. The subsequent LabHEART versions have retained this focus (and we have even developed a student tutorial guide). 2. Our work in dovetailing our model for incorporation into McCulloch's whole heart model constitutes another kind of accessibility that is important (but differs from the stand alone LabHEART). 3. Our newer Shannon-model with additional compartments is also currently being developed both ways (flexible for integration in larger scale models, but also for the stand-alone cellular model).

Research Plans

In the final year we will need to complete many of the ongoing modeling efforts, publish manuscript where appropriate and use them in additional ways. Some key aims are to:

- Complete and publish LabHEART 4.9x and 5.0 versions and make them freely available.
- Complete and publish the new Shannon-model, as well as use it to more fully explore how perturbations in conditions (including rate, adrenergic state) alter electrophysiological and Ca handling properties. Additional perturbations are directly related to ongoing studies by Dr. Beverly Lorell's group where changes in expression of Ca transport and ion channels that occur upon cardiac unloading can be more realistically simulated.
- Connect the Shannon-model to the myofilament ODE model to allow the first up-to-date model combining ion channels, Ca transport and contractile elements (in both variants of user friendliness).
- Extend our collaboration with the whole heart modeling efforts of McCulloch's group which will allow more direct studies of the acute affects of cardiac unloading (as in weightlessness) can be explored (and then observed altered cellular expression of transporters and channels) can be superimposed to simulate more long-term systemic compensations.

Countermeasure Development Plans

This particular project is more tuned to providing a computational platform on which to better understand how changes that occur during spaceflight at the more cellular and molecular level can be understood (and intervened upon) in a more integrated framework. In particular the alterations in expression and function with cardiac unloading described by Lorell's group can be incorporated into our computational model (especially when synthesized into the whole heart context by McCulloch's group) to understand why function is altered and how that may be practically counterbalanced (e.g. by α -adrenergic stimulation or other strategies).

Collaborations

Our group already included collaboration of investigators at four different institutions with complementary strengths (Bers and Puglisi, Loyola University, Chicago; deTombe and Solaro, University of Illinois, Chicago; Shannon at Rush University, Chicago; and Rice, IBM, New York). This has allowed good progress to be made along all of the specific aims originally proposed. Inter-group collaborative relationships have also developed, especially strongly between our group and that of McCulloch, and that has extended the sphere of expertise and impact of both groups with respect to modeling. Additional interactions between our group and that of Lorell's have brought some of the biological consequences more clearly into view, and minor interactions have occurred with other modeling and experimentally focused teams.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Vincent M. Cassone, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Microgravity and Circadian Cardiovascular Function

Project Executive Summary

This project is directed at the physiological mechanism(s) by which the mammalian circadian clock located within the hypothalamic suprachiasmatic nuclei (SCN) regulates cardiovascular function and to what extent simulated microgravity affects circadian variation in cardiovascular function. The interaction of circadian organization and other determinative factors involved in problems associated with microgravity and cardiovascular disease will be assessed through the comparative study of circadian regulation of cardiovascular function in male vs. female rats.

It is known that astronauts suffer many disruptions to normal bodily processes while in space. The most obvious of these is the redistribution of fluids in the body. This was demonstrated as early as the Mercury era, when man first ventured into space. In the microgravity environment, fluids tend to move into the chest and head, causing facial swelling and congestion. This fluid shift also reduces circulating blood volume and plasma levels of norepinephrine as well as causing a specific increased sensitivity of beta-adrenoreceptors. These changes occur due to a rise in blood pressure as perceived by the carotid baroreceptors. In Earth-based studies, bed-rest with head oriented below the feet (HDT) is believed to simulate these effects in space. HDT causes an attenuation of blood pressure rhythmicity, causing damping out of the circadian rhythm of diastolic blood pressure. Systolic blood pressure was not affected as greatly by HDT. However, HDT did not affect the circadian variation in heart rate. However, studies monitoring heart rate while in flight show that heart rate tends to increase after several days in the microgravity environment. While the circadian period of heart rate may not change, there seems to be an increase in heart rate itself.

a) Specific Aim #1: Determination of SCN Efferents Controlling Circadian Variations of Cardiovascular Function in Long-Term, Conscious Rats: Since it is well-known that cardiovascular responses to pressors and stress are significantly different in anaesthetized vs conscious preparations, we will characterize circadian variation in cardiovascular function in conscious freely moving rats. We will then determine whether surgical blockade of SCN efferents affects the circadian variation of heart rate, cardiac output and mean arterial pressure.

b) Specific Aim #2: Role of Circadian System on Daily and Circadian Variation in Regional Blood Flow: We will determine daily and circadian variations in regional blood flow measurements using ⁸⁵Sr-labelled microspheres in rats whose circadian phases will be monitored independently. We will also determine whether 1) the SCN, 2) SCN efferents and 3) sympathetic innervation are required for the expression of these rhythms.

c) Specific Aim #3: Effects of Simulated Microgravity on Circadian Cardiovascular Rhythms: We will determine the effects of hind-limb unloading on the circadian variation in heart rate, regional blood flow and other cardiovascular variables. Based upon data obtained in **Specific Aims #1 and 2**, we will determine the mechanisms by which anticipated changes occur. These experiments will provide guidelines for future counter-measures in space.

d) Specific Aim #4: Effects of Gender on Circadian Changes in Cardiovascular Function and Their role in Responses to Microgravity: Because it is well-established that female and male astronauts experience a different set of cardiovascular responses to microgravity, we will also determine whether we can simulate those differences in our simulated microgravitational apparatus. If so, we will employ the information gained in **Specific Aims #1 and 2** to provide guidelines for future countermeasures.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Richard J. Cohen, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Cardiovascular Effects of Simulated Microgravity in Man

Project Executive Summary

This project is targeted towards studying and developing countermeasures to two of the Cardiovascular Critical Risks:

- (i) Development of post-flight orthostatic intolerance
- (ii) Increased susceptibility to ventricular dysrhythmias.

The development of orthostatic intolerance is a well known adverse effect of space flight on the cardiovascular system, and is a current operational problem for NASA. Astronauts post-flight may experience a drop in arterial pressure upon adopting the upright posture after flight, which may be sufficiently severe to cause presyncope or syncope. This effect is greater the longer the duration of the flight, and is more pronounced in women than in men. During space flight intravascular volume is decreased and cardiovascular reflexes are down-regulated because the cardiovascular system is no longer subjected to the stresses associated with changes in posture. Upon return to a gravitational environment, blood pools in the large veins of the lower extremities and the splanchnic circulation, leading to a drop in preload to the heart leading to a decrease in cardiac output. In addition, the reflex ability to increase heart rate and constrict arteries and veins is diminished, and there are also changes in cardiac systolic and diastolic function. Countermeasures of salt and water loading prior to re-entry and the use of G-suits are not adequate countermeasures to prevent the development of orthostatic intolerance, particularly after long duration flights. Our goal with respect to this cardiovascular risk is to better understand the detailed mechanisms leading to orthostatic intolerance and to develop and test mechanism based countermeasures.

There have been several anecdotal reports of documented episodes of self-terminating ventricular tachycardia during space flight¹⁴. In addition it has been reported that Russian cosmonauts have suffered from ventricular arrhythmias, and two primates have suffered cardiovascular collapse after return from space flight (without ECG documentation). These data suggest that space flight may be conducive to the development of ventricular arrhythmias. However, it is not known whether or not this is in fact the case. If long duration space flight does increase the risk of potentially lethal ventricular arrhythmias then this would obviously pose an enormous problem for very long duration flights. Our goal in this project is to determine in controlled ground based experiments, whether there is evidence that simulated micro-gravity in fact alters cardiac electrical activity in a manner that may increase susceptibility to ventricular arrhythmias. If we find evidence that this is in fact the case, then we will attempt to establish mechanism and identify potential countermeasures.

In this project we analyze data from ground based human studies in which 16 day head down tilt bed rest is used to simulate microgravity. We will be studying the following groups in order to examine the effects of gender and age on these cardiovascular risks.

- i. men under age 50
- ii. premenopausal women
- iii. men over age 50

In addition to the effects of bed rest we will also examine the effects of sleep deprivation (another condition of space flight). We will also evaluate the effects of the alpha-agonist midodrine as a countermeasure to the development of orthostatic intolerance. We may also evaluate a countermeasure to the development of ventricular arrhythmias.

The key technologies we will utilize are Cardiovascular System Identification (CSI) as a noninvasive means of assessing closed-loop cardiovascular regulation, and measurement of microvolt T-wave alternans (MTWA) as a noninvasive measurement of changes in cardiac repolarization which has been shown in clinical trials to be an accurate measure to susceptibility to ventricular arrhythmias.

To date in this project, we have found that CSI measures of autonomically mediated cardiovascular reflexes are diminished by bed rest, and that during bed rest there appears to be a shift towards increasing sympathetic/parasympathetic balance. We have found that pre-bedrest CSI measures of increased sympathetic/parasympathetic balance identify those subjects who tolerate tilt both pre-bedrest and post-bedrest. We have put considerable effort into improving CSI technology for use in these studies and for application in biomedical research and patient monitoring.

We have demonstrated that the alpha-agonist midodrine appears to be an effective countermeasure to the development of orthostatic intolerance after exposure to 16 days of simulated microgravity.

We have found that even 16 days of bed rest tends to induce sustained MTWA although not at a level that would be of immediate clinical concern. This evidence does indicate that bed rest does measurably alter cardiac repolarization processes, and raises the issue of whether long term exposure to microgravity could increase susceptibility to ventricular arrhythmias. We have also recently conducted a clinical study that demonstrates that MTWA is an accurate predictor of susceptibility to ventricular arrhythmias and sudden cardiac death in patients with heart failure and no prior history of sustained ventricular arrhythmias.

We plan to continue our ground based studies in the above identified patient groups, and evaluate the effects of sleep deprivation and midodrine countermeasures. We may also test a proposed countermeasure to the development of ventricular arrhythmias pending future results from these studies. In addition, we have submitted a flight proposal to measure CSI and MTWA pre and post flight, and to evaluate the midodrine countermeasure during flight conditions.

We plan to develop further the CSI and MTWA technologies for use on earth for biomedical research and clinical applications.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Richard J. Cohen, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Effects of Space Flight on Cardiovascular Stability

Project Executive Summary

Many astronauts after being weightless in space become hypotensive and presyncopal upon assuming an upright position. This phenomenon, known as orthostatic intolerance, may interfere with astronaut function during reentry and following space flight, and may limit the ability of an astronaut to exit a landed spacecraft unaided during an emergency. Orthostatic intolerance is more pronounced following long-term space flight and is a major concern with respect to the extended flights expected aboard the International Space Station and for interplanetary exploration class missions, such as a human mission to Mars. This problem has also been observed to be more pronounced among women than among men. In addition to the problem of post-flight orthostatic intolerance, a variety of heart rhythm disturbances have been observed in astronauts during and after space flight. The potential lethal arrhythmic risk for astronauts is sustained ventricular tachycardia or ventricular fibrillation, while non-sustained ventricular tachycardia could cause syncope.

In previous ground based bed rest studies sponsored by NSBRI we have applied two new techniques that we have developed to study the effects of simulated microgravity on the cardiovascular system. Cardiovascular system identification (CSI) has been used a non-invasive means of measuring alterations in closed-loop cardiovascular regulation and the measurement of microvolt level T wave alternans (TWA) has been used as a non-invasive measure of susceptibility to ventricular arrhythmias. We have also successfully tested the alpha-1 sympathetic agonist midodrine as a countermeasure to the development of orthostatic intolerance. We have found that 16 days of bed rest results in altered cardiovascular regulation. In particular, we have demonstrated alterations in baroreceptor sensitivity, altered electrical stability of the heart, and that midodrine is an effective countermeasure to the development of orthostatic intolerance.

In this proposal we plan to apply the same measurement techniques of CSI and TWA to astronauts pre- and post-flight and to test midodrine as a countermeasure to the development of orthostatic intolerance. This study will allow us to determine if the changes in cardiovascular regulation and cardiac electrical stability measured in a ground-based model also occur during actual space flight. In addition we will test for the first time a potentially highly effective countermeasure for the development of post-flight orthostatic intolerance.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	James E. Coolahan, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Distributed Simulation of Integrated Human Function

Project Executive Summary

The project Distributed Simulation of Integrated Human Function is being undertaken for the National Space Biomedical Research Institute (NSBRI) by the Johns Hopkins University (JHU), under the leadership of the Applied Physics Laboratory (APL) in collaboration with the Whitaker Biomedical Engineering Institute and the Center for Computational Medicine and Biology. In 2002, upon the dissolution of the NSBRI Integrated Human Function team, the project was transferred to the NSBRI Cardiovascular Alterations team. This report documents the accomplishments during the second award year of the project, which was actually a 14-month period from January 1, 2002, through February 28, 2003.

Review of Specific Aims

The four specific aims of the three-year project, taken from the project proposal, are as follows:

1. To develop, at JHU, an experimentally-based computational model of the human ventricular myocyte using cells isolated from tissue biopsies performed in patients; and to develop a finite-element model of the geometry and fiber structure of the human heart using diffusion-tensor imaging data to be collected at JHU, fit by a finite-element model to be developed at the University of California, San Diego (UCSD);
2. To develop a distributed simulation of human cardiac function, incorporating a simulation of the human cardiac ventricular cell resident at JHU based on the model discussed above and a simulation of coupled cardiac mechanical and electrical function resident at UCSD, with distributed simulation control based at JHU/APL;
3. Working with the NSBRI Integrated Human Function (IHF) Team, to select other appropriate cardiovascular system models that can be represented over time using simulations, and integrate them into a distributed simulation of cardiovascular function; and
4. Again working with the NSBRI IHF Team, to select bone and muscle models that can be represented over time using simulations, and integrate them into a multi-function distributed simulation representative of the full IHF simulations that will be needed for long-duration space flight.

Key Project Findings in the Second Award Year

In the second award year, we have made progress on three of the four specific aims. On specific aim one, in the Winslow Laboratory at JHU, the development of the human ventricular myocyte model has been completed. The model accurately describes properties of voltage-gated ion currents identified in human ventricular myocytes, including: Na current; L-type Ca current; delayed rectifier currents I_{kr} and I_{ks} ; transient outward K current I_{to1} ; and instantaneous inward rectifier current I_{k1} . The resulting model can reproduce the following properties of human ventricular myocyte action potentials: action potential shape; AP duration changes as a function of pacing frequency; and extra-systolic restitution and post-extrasystolic potentiation curves. Unlike many myocyte models, this model achieves Na equilibrium across a broad range of pacing frequencies. Concerning our image-based models of the heart, during the second award year, we have continued to have difficulties in obtaining human heart specimens. To resolve

this, we have written a grant application to the National Disease Research Interchange requesting that we be provided with six transplant-grade human hearts. Our application was approved in February 2003. We are now “on-call” to receive these hearts at a moment’s notice. In the interim, we have made substantial advances in the spatial resolution that can be achieved in Diffusion Tensor Magnetic Resonance Imaging (DTMRI) through experiments performed on canine hearts, in which we have developed a novel 3-D diffusion imaging protocol.

We reported progress along two fronts on specific aim two in the annual report for the first award year. As this aim’s primary intent was to demonstrate the ability of the High Level Architecture (HLA) to allow biomedical simulations to interoperate (which we achieved in the first year through the Cardiovascular-Ventricular Simulation (CVVS) federation under specific aim three), and recognizing the NSBRI intent to focus on multi-system model integration, we deferred work on specific aim two during the second award year in favor of increased progress on specific aim four.

On specific aim three, during the first award year, in order to examine the connection between arrhythmias and blood flow, we initiated a collaboration with members of Dr. Roger Mark’s group (NSBRI Cardiovascular Alterations team) at the Massachusetts Institute of Technology (MIT). We integrated, using the HLA, a medium-fidelity two-dimensional electrical-mechanical simulation of the left and right ventricles (the Hybrid Cellular Automata (HCA) heart simulation) with a cardiovascular system model (the Research Cardiovascular Simulator (RCVSIM), developed at MIT) to form the CVVS federation. During the project’s second award year, we advanced our cardiovascular federation along two fronts: conversion of the CVVS federation to the IEEE 1516 HLA standard, and extension of the HCA heart simulation to three dimensions.

Since the development of the original CVVS federation, the HLA has completed the transition from a U.S. DoD standard to an IEEE standard. We re-implemented the CVVS federation using the IEEE 1516 HLA RunTime Infrastructure (RTI) software from the Pitch AB software company (Linköping, Sweden). The transition to the IEEE 1516 HLA specification went smoothly, and has offered numerous practical advantages, including more consistent and precise language throughout, increased type safety to allow for improved and faster debugging, construction of arbitrarily complex data types using the new Object Model Template (OMT), more automated configuration during installation, and increased portability by use of the Java programming language. Our IEEE 1516 HLA-compliant CVVS federation was determined to be the first HLA federation employing this new standard in any application in the U.S.

During the second award year of the project, we have furthered the development of the medium-fidelity HCA model of cardiac electrical conduction on two fronts: physiologically-realistic simulation of front propagation in cardiac muscle with twisting fiber structures using finite elements; and whole-heart implementations of this model using approximate models of the real 3-D human ventricular geometry. The equations for local propagation along cardiac fibers in the HCA model were first derived for an isotropic 3-D cardiac medium, and then the resulting model parameter values were locally modified using scaling relations to reflect the anisotropies arising from the local fiber orientation. In the first set of simulation studies, we calculated propagating wave fronts using a 5 cm x 5 cm x 1 cm 3-D domain representing a slab of ventricular muscle. We also performed an initial set of numerical experiments using a fully 3-D approximate model of the human ventricles. The model contains 2.8 million HCA elements and has a left ventricular volume of approximately 108 ml. We also calculated the Q-T interval dynamics for our model to show the changes in electrocardiogram (ECG) morphology and Q-T interval associated with an abrupt change of heart rate from 60 beats per minute (BPM) to 120 BPM.

On specific aim four, in response to the NSBRI desire to focus its integrated modeling effort on the goal of producing a simulation of human exercise, we began a collaboration with Dr. Marco E. Cabrera (Case Western Reserve University; NSBRI Nutrition, Physical Fitness and Rehabilitation team) and Dr. Martin J. Kushmerick (University of Washington; NSBRI Muscle Alterations and Atrophy team). Building upon a streamlined version of our CVVS federation, we have made significant progress in constructing an expanded federation incorporating a blood flow federate based on work done at MIT, Dr. Cabrera's whole-body lactate metabolism model, Dr. Kushmerick's skeletal muscle energetics model, several smaller specialized federates, and a data collection federate. The intent of this federation is to simulate human exercise on a cycle ergometer, following the exercise protocol used by U. S. astronauts. We are currently in the final integration and testing stages of this human exercise federation (ExerFed).

Impact of Findings on Hypothesis, Objectives and Specific Aims

The findings of the project during the first award year supported the hypothesis that interoperable simulations of human physiological functions applicable to the space flight environment, executing interactively, can produce integrated results that cannot be produced by these simulations executing independently. We have re-enforced our conclusions in the second year by our collaborative work with Drs. Kushmerick and Cabrera in extending the federation to incorporate simulations of whole-body metabolism and skeletal muscle energetics.

The progress on the simulation of human exercise during the second award year has significantly contributed to the long-term project objective to demonstrate the ability to simulate integrated human function over time by providing a technical framework to permit simulations of different human physiological functions, executing in separate locations, to interact to produce synergistic results. Although we are currently executing a preliminary version of the federation over a local area network at JHU/APL, we intend to demonstrate the capability for distributed execution during the third award year.

Proposed Research Plan for the Third Award Year

In the third award year of the project, we plan to continue work toward achieving the original specific aims, with continued concentration on aims one, three and four.

We plan to continue and complete work in the Winslow Laboratory on the imaging of human hearts, in order to produce the human heart geometry needed for the full human heart model.

We also intend to continue work on the 3-D HCA heart model. When used in conjunction with a simple model for the local action potential, this modeling approach can be used to provide activation sequences and relaxation dynamics for driving mechanical models of the pumping of the heart. We intend to examine this integration with our collaborator, Dr. Andrew McCulloch at UCSD. Integration of a combined, computationally-efficient, electrical-mechanical simulator with a cardiovascular system model, such as RCVSIM, in a revised version of our CVVS federation will permit simulation and analyses of hemodynamics associated with specific rhythm disturbances under both normal conditions and altered physiology associated with microgravity.

Finally, we intend to continue development of the HLA-compliant human exercise federation, working in collaboration with Drs. Cabrera and Kushmerick. We intend to test and refine the simulation using cycle ergometer data collected in a laboratory environment. Once we are satisfied with results using this data, we intend to seek both ground-based and, ultimately, space-based data from U.S. astronaut exercise protocols using the cycle ergometer.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Michael D. Delp, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Circulatory Remodeling with Simulated Microgravity

Project Executive Summary

Research Program Structure and Design

The proposed studies were designed address the effects of simulated microgravity on the arterial and lymphatic portions of the circulation. Using the hindlimb unloaded rat as a ground-based animal model, the general aim of this proposal is to determine the effects of simulated microgravity on 1) the molecular mechanisms mediating structural remodeling of the arterial resistance vasculature, and 2) the ability of the lymphatics to generate and modulate lymph flow. The following Specific Aims and hypotheses were proposed in this grant.

Aim 1

To identify early regulatory events leading to hypertrophic remodeling of cerebral arteries in response to hindlimb unloading. We will characterize indicators of cell proliferation to determine whether increased medial thickness previously reported is due to cell growth. Utilizing RT-PCR, in situ hybridization and immunohistochemistry, we will identify key signaling mediators that are involved in this process. Our initial studies will concentrate on nitric oxide (NO) and endothelin (ET), both of which have been proposed to mediate mechanically induced vascular remodeling in other systems. Key cell growth, survival and differentiation markers of vascular smooth muscle cells will be examined to better determine their role in hypertrophic remodeling of the cerebral arteries.

Aim 2

To characterize signaling events leading to atrophy of resistance arteries in the soleus and gastrocnemius muscle in response to hindlimb unloading. Resistance arteries in the soleus muscle have been shown to atrophy in response to hindlimb unloading in a manner that leads to decreases in luminal diameter as a result of circumferential atrophy of smooth muscle cells (i.e., a decrease in muscle length). Intraluminal shear stress has been shown to decrease during the initial unloading period, but as remodeling occurs, shear stress returns to control levels. The goal of this aim will be to determine if there is increased susceptibility to apoptosis in smooth muscle cells with reduced shear, to evaluate nitric oxide synthase (NOS) activity and expression, and to determine if activation of matrix metalloproteinase activity is up regulated and contributes to decreased luminal diameter. In contrast to the apparent shear stress-mediated remodeling of soleus muscle resistance arteries, reduced transmural pressure appears to be the primary stimulus for remodeling of resistance arteries from the gastrocnemius muscle. The hindlimb unloading-induced remodeling of the gastrocnemius muscle resistance arteries does not involve alterations in the vessel diameter, but rather consists of a decrease in media thickness that appears to occur as a result of radial atrophy of smooth muscle cells (i.e., decreased thickness of smooth muscle cells). We will utilize cellular markers of apoptosis to quantify susceptibility to cell death. RT-PCR, in situ hybridization and immunohistochemistry will be used to quantify expression of growth factors, survival factors and contractile proteins in these vessels in response to hindlimb unloading.

Aim 3

To evaluate the effects of hindlimb unloading on the ability of the lymphatics from different regions of the body to generate and modulate lymph flow, and thus, regulate overall body fluid homeostasis. It has been demonstrated that acute change in the transmural pressure, luminal flow and outflow resistance will modulate lymph contractile activity and lymph flow. Furthermore, it is known that the special contractile characteristics of the lymphatics are reflected in the expression of contractile proteins within the lymphatic muscle cells. Given that hindlimb unloading induces tissue fluid shifts from the lower to the central and upper portions of the body, it is likely that these tissue fluid shifts will differentially alter the normal physical environment of the lymphatics in different lymphatic drainages. Thus, similar to that found in the arterial circulation, we hypothesize that chronic changes in the normal physical environment of the lymphatics (i.e., changes in tissue fluid pressure, lymph pressure and central venous pressure) will alter both the lymphatic contractile function and the expression of contractile proteins within the lymphatic muscle. Specifically, we will evaluate lymphatic contractile function and contractile protein expression from five regions of the body where significant fluid shifts are known to occur in response to microgravity, as well as in tissues where the majority of the body's lymph is produced.

The present proposal is synergistically related to several projects within the cardiovascular team:

1. The current project PI (Michael Delp) will assist the PI of another NSBRI cardiovascular project, Dr. Vince Cassone, with studies to determine the effects of hindlimb unloading on circadian changes in cardiac output and blood flow distribution.
2. The current studies determining the effects of simulated microgravity on the arterial and lymphatic portions of the circulation are complimentary to those of Drs. Artin Shoukas and Dan Berkowitz, who will investigate the effects of hindlimb unloading on the venous portion of the circulation. Thus, these projects will provide a comprehensive investigation of the peripheral circulation in hindlimb unloaded rats.
3. A collaboration between the current project PI (Michael Delp) and the PI of another NSBRI cardiovascular project, Dr. Chester Ray, was established to determine the effects of microgravity and hindlimb unloading on cardiac mass in rats. This project was funded, in part, by both of the current NSBRI grants to these PIs, and the results have been recently published.

Research Program Accomplishments

The initial studies were designed to determine the mRNA and protein expression of endothelial nitric oxide synthase (eNOS) in cerebral and skeletal muscle resistance arteries, which has been proposed to mediate mechanically induced vascular remodeling in other systems (Specific Aims 1 and 2). In the cerebral arteries, eNOS mRNA expression was not different between control and hindlimb unloaded rats after 1, 14 and 28 days of hindlimb unloading. However, eNOS protein levels are significantly depressed in the middle cerebral artery (MCA) after 14 days of hindlimb unloading.

The current results indicate that eNOS may be directly involved in the remodeling of the cerebral vascular hypertrophy induced by hindlimb unloading in rats. These results were presented at the Humans in Space Symposium, and a manuscript is in preparation.

Lymphatic contractile function from the mesenteric lymphatics and thoracic ducts have been tested and characterized from control rats, and the results have been published. Furthermore, the effect of hindlimb unloading has been determined to diminish the contractile function in these lymphatic vessels. More specifically, there is a 50-75 percent reduction in resting tone of lymphatic vessels, a 30-60 percent reduction in phasic contraction frequency of the lymph pump, a 60-80 percent reduction in the strength of phasic contractions of the lymph pump, and a significant reduction in the pressure-sensitive stimulation of the lymph pump. These results of simulated microgravity have been presented at the Bioastronautics Meeting and the Humans in Space Symposium. A manuscript also is in preparation.

One question in the cardiovascular area has been whether microgravity induces cardiac atrophy. We recently reported that results from rats flown for one week on the Spacelab 3 mission demonstrate that cardiac atrophy does not occur with short-term exposure to microgravity in rats (Ray et al. *J Appl Physiol* 91: 1207-1213, 2001). Similarly, we found that neither one week nor four weeks of hindlimb unloading induced cardiac atrophy or altered the peak rate of rise in left ventricular pressure, and index of myocardial contractility (Ray et al. *J Appl Physiol* 91: 1207-1213, 2001). However, there are studies in the literature reporting cardiac atrophy in hindlimb unloaded rats. To determine whether the cardiac atrophy reported in the literature may be related to caloric deficits in some hindlimb unloaded rats, we plotted heart mass as a function of body mass from all studies reporting these variables in the literature. We found that in all cases where cardiac atrophy was reported, there was a substantial corresponding loss of body mass. Therefore, these findings indicate that cardiac atrophy and dysfunction are not adverse consequences of short-term microgravity or long-term simulations of microgravity when body mass is fairly well maintained.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Beverly H. Lorell, M.D.
ORGANIZATION:	Harvard – Beth Israel Deaconess Medical Center
PROJECT TITLE:	Cardiac Unloading: Biologic Mechanisms and Countermeasures for Cardiac Atrophy

Project Executive Summary

Project Aims

The aims of the project are to determine functional consequences of cardiac remodeling due to microgravitational unloading using earth-based model of heterotopic transplantation. The following biologic effects of this surrogate model of cardiac unloading will be examined:

1. Effects on adult myocyte contractile function and Ca²⁺ regulation Regulation of myocyte growth and programmed cell death (apoptosis)
3. Identification of human-relevant countermeasures which blunt cardiac atrophy and/or enhance functional cardiac reserve (including alpha-adrenergic agents).

Key Findings

The key findings of the project to date are the following:

A. Using the heterotopic transplant model of cardiac unloading, we made three observations:

1. Cardiac unloading modifies contractile reserve of cardiac myocytes, ie, the ability of heart muscle cells to do extra work.
2. The biologic mechanism is related to a distinct “molecular signature” the expression of Ca²⁺ regulatory genes in the heart.
3. The changes are related to magnitude and duration of unloading. These observations have direct implications for planning future human studies, and suggest that lessons from short-term spaceflight may not necessarily predict biologic effects of long-term spaceflight in the hearts of astronauts.

This work is in press in *Circulation*, 2003, pending final requested revisions.

B. Using genetic mouse models, two novel pathways for preservation of cardiac mass and function have been identified:

1. Cyclin-dependent kinase-9 pathway
2. Telomerase reverse transcriptase pathway.

Impact of Findings

The key findings of the project to date directly confirm the hypothesis of specific aim 1: Cardiac unloading *does* affect both cardiac myocyte contractile function and Ca²⁺ regulation.

Proposed Research Plan for the Coming Year

In the coming year, experiments will focus on specific aim 3: Countermeasure development. We will perform studies to test the hypotheses:

1. Cardiac unloading modifies cardiac adrenergic receptor signaling
2. Therapy aimed at α -adrenergic receptor stimulation may serve as a countermeasure for improvement of cardiac reserve of the heart.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Roger G. Mark, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Computational Models of the Cardiovascular System and its Response to Microgravity and Disease

Project Executive Summary

One of the highest priority problems in the current manned space program is orthostatic intolerance experienced by astronauts upon their return to the normal gravitational environment. This problem has been well known since the earliest days of manned spaceflight, and has been intensely investigated in in-flight studies and in many land-based simulation (bed-rest) studies. A number of countermeasures have been proposed and evaluated, but no effective and practical countermeasure has been developed to date. The number of hypotheses still under consideration and the lack of a single unifying theory of the pathophysiology of orthostatic intolerance testify to the difficulty of the problem.

Computational models of the cardiovascular system can help in this situation in that they provide a rational framework that quantitatively defines interactions among complex cardiovascular parameters and supports the clinical interpretation of experimental results and testing of hypotheses. Models also permit predictions of the impact of specific countermeasures in the context of various hypothetical cardiovascular abnormalities induced by microgravity.

These same models may also play a useful role in clinical medicine, for example, by improving the organization and interpretation of multi-parameter physiologic data in intensive care units, and the tracking of a patient's status over time. The model being developed in this research, although aimed primarily at the operational problem of microgravity-induced orthostatic intolerance, therefore has important potential clinical applications.

This project will develop a general, modular model of the cardiovascular system that contains the essential features associated with the effects of gravity, and will use this model to examine the short-term hemodynamic response of the cardiovascular system to abrupt orthostatic transitions. The model will facilitate the understanding of the physiology and treatment (prevention) of OI in post-flight astronauts. We will extend the progress already made over the past years, with the following specific aims:

1. Verify the model, and use it to a) investigate and critically evaluate various hypotheses for orthostatic intolerance by matching simulations to appropriately chosen experimental data and b) predict the effects of countermeasures and various orthostatic stresses experienced in space flight and during ground-based experiments. This specific aim is of major importance, and requires:

- Collection of extensive experimental measurements from human studies performed by a number of collaborating investigators.

- Reformatting the data according to standards used in our extensive NIH-supported archives (www.physionet.org).
 - Storing the data for our use and also for the use of other investigators as appropriate.
2. Enhance the current version of our cardiovascular simulation to better represent the short-term effects of abrupt orthostatic stress. Specifically, we will: a) consider the addition of vasoactive hormone loops to the control system to account for the rapid actions of norepinephrine, epinephrine, renin-angiotensin-II, and ADH (vasopressin); b) add threshold/saturation characteristics, latencies, and dynamics to the individual sympathetic effector limbs, adding a term to the baroreceptor response proportional to the rate of change of arterial pressure, and modify the arterial baroreflex to account for the aortic baroreceptors; c) add atria to the cardiac model to enhance stroke volume at high heart rates.
 3. Develop quantitative techniques for comparing simulations and experimental data and develop methods for searching the model's parameter space to identify sets of parameters that best fit a given experimental recording.
 4. Complete, document and disseminate to other investigators the JAVA version of the cardiovascular simulator. Prepare user manuals for both the Linux version of the simulator and the JAVA version.
 5. Apply the cardiovascular model to the clinical problem of intelligent patient monitoring both in the context of intensive care and in tracking chronic cardiovascular disease such as congestive heart failure. This specific aim will require the acquisition of an enhanced research database of multi-parameter hemodynamic data from intensive care unit patients.

Major progress has been made during the past fiscal year of the program:

1. We have simulated the hemodynamic response to sudden-onset exercise (bicycle ergometry) in a contribution to the NSBRI Integrative Human Function Core workshop in Seattle, Washington.
2. We have enhanced the cardiovascular reflex model by introducing an aortic arch baroreflex and by allowing for reflex impulse response functions to be individualized for each vascular bed.
3. We have developed and implemented techniques that allow for automated matching of simulation output to sets of experimental data.
4. We have investigated the transient hemodynamic response to active and passive changes in posture to validate the model's predictions.
5. We have gathered, formatted, and archived in standardized form tilt and stand test data from the NSBRI bed rest studies, astronaut tilt and stand test data from the cardiovascular lab at Johnson Space Center, and orthostatic stress data from collaborating investigators. This data is currently being used for model verification and testing of hypotheses regarding the mechanisms of orthostatic intolerance.

6. We have begun exploring model reduction methodologies that allow for the adaptation of model structures to the characteristics of available physiologic data.
7. We publicized and made freely available on the web a public version of the cardiovascular simulator (<http://www.physionet.org/physiotools/rcvsim>).

The research presented in this progress report is well within the time frame set forth in the original proposal and the progress made so far does not necessitate any changes to the specific aims of the proposal or the strategy with which the research is pursued.

We intend to pursue the following goals during year three of the funding period:

1. Continue to gather data from collaborators, convert the data obtained into standard format, archive the data on our central server, and incorporate the data into our analysis of the transient hemodynamic response to stand/tilt and LBNP.
2. Continue to apply the parameter estimation routine to experimental data from normal and microgravity adapted individuals.
3. Investigate the physiologic difference between active and passive changes in posture and the information content these interventions provide.
4. Apply the cardiovascular model to the clinical problem of patient monitoring both in the context of intensive care and in tracking chronic cardiovascular disease.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Andrew D. McCulloch, Ph.D.
ORGANIZATION:	University of California, San Diego
PROJECT TITLE:	Integrated Modeling of Cardiac Mechanical and Electrical Function

Project Executive Summary

Project Aims

Aim 1: To apply our existing techniques for modeling three-dimensional cardiac mechanics and action potential propagation to develop anatomically detailed three-dimensional dynamic finite element models of regional cardiac electromechanics.

Aim 2: To bridge models and data on cardiac metabolism and cellular dynamics with systems models of coronary flow, central hemodynamics, and cardiovascular regulation.

Aim 3: To develop tools for using available wall motion data from medical imaging in man to validate the mechanoenergetic models and identify myocardial constitutive properties.

Aim 4: To apply new models of geometric and constitutive remodeling in response to chronically altered external loading conditions to develop simulations of long-term cardiac adaptation to microgravity.

Aim 5: To implement the models using modular object-oriented software engineering techniques that allow the models to be readily integrated with others through standard broker architectures for software interoperability.

Aim 6: To collaborate with other prospective projects in the Integrated Human Function Core.

Key Findings/Progress

Significant progress has been made coupling cardiac electromechanical models. Two new papers have been accepted applying and extending the a new computational model reported last year (Usyk TP, LeGrice IJ, McCulloch AD. Computational model of three-dimensional cardiac electromechanics. *Comput Visual Sci* 4(4):249-257, 2002.) in which a model of anisotropic cardiac impulse propagation is coupled to a model of three-dimensional anisotropic ventricular wall mechanics in an anatomically detailed three-dimensional model of the left and right ventricles that also includes a model of the Purkinje fiber network anatomy. In one new paper now in press (Usyk, T. P. and A. D. McCulloch. Relationship between regional shortening and asynchronous electrical activation in a three-dimensional model of ventricular electromechanics (*Journal of Cardiovascular Electrophysiology* 14 (suppl.)) this model has been used to investigate the effects of altered mechanical pacing sequence due to ectopic activation, and showed excellent agreement with published experimental data. In another paper now in press (Usyk, T. P. and A. D. McCulloch. Electromechanical model of cardiac resynchronization in the dilated failing heart with left bundle branch block. *J Electrocardiol*, 2003.), we tested the ability of the model to predict countermeasure effectiveness, by using it to predict the effects of bi-ventricular pacing for cardiac resynchronization therapy in an experimental model of heart failure with a conduction defect. Again the model predictions showed very good agreement with experimental measurements.

As a result of the NSBRI Exercise workshop in Seattle in September 2002, models of cardiac electromechanics during exercise are focussing on the effects of heart rate, adrenergic stimulation and acidosis. We have developed a new model of adrenergic signaling in the cardiac myocyte that couples to electrophysiology by simulating the effects of β adrenergic receptor stimulation on excitation-contraction coupling mechanisms via phosphorylation of several cellular targets by protein kinase A. This new paper now in press (Saucerman, J. J., L. L. Brunton, A. P. Michailova, A. D. McCulloch. Modeling beta-adrenergic control of cardiac myocyte contractility in silico. *J Biol Chem*, 2003.) successfully recapitulates major effects of the neurohormonal activation that occurs during exercise on the ventricular myocyte.

A new object-oriented modular version of *Continuity* that was released during the previous period has been updated. A new release, tentatively named release 6.2 is due for release in the Fall 2003. It has a modular client-server design, very high-level scripting language, a graphical user interface and new three-dimensional viewer. The newest version supports additional computer platforms, especially Linux. It has also been used successfully in classroom teaching.

We have engaged in productive collaborative activities with other members of the Integrated Human Function Core, especially the group of Dr. Bers. As the result of discussions with the Cardiovascular Alterations Team, we have conducted preliminary experiments on changes in restitution dynamics with mechanical loading. This will provide a basis for new computational models of mechanoelectric feedback as a follow-up to our recent report showing how altered mechanical loading slows action potential propagation and prolongs repolarization in the whole heart (Sung D, Mills RW, Schettler J, Narayan SM, Omens JH, McCulloch AD. Ventricular filling slows epicardial conduction and increases action potential duration in an optical mapping study of the isolated rabbit heart. *J Cardiovasc Electrophysiol* 14(7):739-749, 2003.)

Impact

These findings of the three-dimensional electromechanical models validate the original premise of the proposal that an integrated cardiac three-dimensional electromechanical model is both computational feasible and physiologically predictive. They also demonstrate the utility of such models for countermeasure design, assessment and validation. This is a fundamental advance that paves the way for the other objectives and applications. By including cell signaling pathways in the cellular models of cardiac excitation-contraction coupling, we will be in a position to simulate the physiological effects of exercise and other stresses that activate the major pathways that respond to neurohormonal signals in the heart.

The new software, *Continuity 6.0* provides a problem solving environment for integrative modeling that is general enough for both structurally integrated models that couple from single cell to organ system scales and functionally integrated models that couple electrophysiology, mechanics, metabolism and regulatory processes. The methods are generic and thus applicable to other systems such as soft tissues and muscle.

Proposed Research Plan

We will extend the electromechanical models to include more detailed cellular biophysical models of cardiac excitation and contraction whose parameters therefore have greater physiological meaning thus making the models more inherently predictive. These cellular models are developed by our group and the group of Dr. Bers including new models of ionic currents, excitation-contraction coupling, myofilament activation and crossbridge interactions. The integrative whole heart models that contain these improved cellular models will also be used to

incorporate the effects of regional cellular heterogeneity in the heart walls. For example, the T-wave of the electrocardiogram may be an important predictor of potentially life-threatening cardiac events in space. The morphology of this waveform is directly influenced by the fact that myocyte repolarizing currents are different as a function of transmural position in the ventricular wall. We have therefore begun a new model in collaboration with Dr. Bers lab that includes three transmural cell layers in the epicardium, M-cell layer and endocardial regions of the ventricular walls. We will also extend the current cellular models model to include the pH regulation of intracellular calcium handling, the regulation of cardiac electrophysiology and contraction by beta adrenergic stimulation, and the effects of magnesium which may offer a potential countermeasure. The next major release of *Continuity* will include improved facilities for composing and integrating cellular models and for the efficient parallel solution of large-scale whole organ models. Synergistic collaborations with other NSBRI projects will be continued.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Janice M. Meck, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Mechanisms of Post-Space Flight Orthostatic Intolerance

Project Executive Summary

There is still a significant number of astronauts at Johnson Space Center who suffer from post-flight orthostatic hypotension and presyncope. The mandatory use of fluid loading with salt tablets and water, anti-gravity suits, and the liquid cooling garment has not eliminated the problem. A growing body of evidence suggests that there are major physiological systems that become dysfunctional as a result of space flight. The degree of dysfunction varies from minimal to severe. Several studies have provided evidence that autonomic function is impaired during and after space flight. Additional factors such as local factors, could also be involved. An area that has not been studied in humans is the effects of space flight on nitric oxide physiology and its modulation of blood pressure. The study proposed in this application will continue the pursuit of mechanisms of autonomic dysfunction in presyncopal astronauts. In addition, it will begin to elucidate changes in nitric oxide production and the resulting effects on the cardiovascular system. This study will not have in-flight measurements. All procedures will be performed before launch, on landing day and three days after landing. The study has two specific aims: 1) to compare pre-flight to post-flight changes in responses of veins to adrenergic agonists between presyncopal and nonpresyncopal astronauts; 2) to compare pre-flight to post-flight changes in nitric oxide levels, inducible nitric oxide synthase messenger RNA and protein, cell adhesion molecules associated with endothelial activation, responses to acetylcholine with and without nitric oxide synthase inhibition, and reactive hyperemia responses in the brachial artery, the arm and the popliteal artery in the leg, between presyncopal and nonpresyncopal astronauts. Presyncopal and nonpresyncopal astronauts will be defined by their ability to complete a 10-minute upright tilt test on landing day.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Ferid Murad, M.D., Ph.D.
ORGANIZATION:	The University of Texas Health Science Center at Houston
PROJECT TITLE:	A Soluble Guanylyl Cyclase Mouse Knock-Out Model

Project Executive Summary

Specific aims of the project:

Specific Aim 1: To gain an understanding of the sGC-cGMP regulatory pathway in the cardiovascular system by developing an animal model with myocardium-specific and vascular smooth muscle-specific disruption of sGC gene expression. Hemodynamic consequences that result in the elimination of sGC activity in knockout mice versus wild type mice will be assessed.

Aim 1a: To produce *Lox*-targeted cardiac-specific β_1 -sGC knockout mice utilizing standard recombinant cloning and transgenic techniques. Mice will be mated with cardiac specific *Cre* mice (available through Michael Schneider at Baylor College of Medicine, Houston, TX) to obtain the double *Cre/Lox* cardiac-specific β_1 -sGC transgenic progeny and smooth muscle specific *Cre* mice (available through Franz Hofmann at Institut fur Pharmakologie und Toxikologie, Munich, Germany) to obtain the double *Cre/Lox* smooth muscle specific β_1 -sGC transgenic progeny.

Aim 1b: To characterize the effects of myocardial-specific and vascular smooth muscle-specific sGC gene disruption on general appearance, ECG, arterial blood pressure, heart rate, cardiac Doppler measurements, embryonic development and histology of the heart in knockout mice versus wild type mice.

Specific Aim 2: To determine the role of the sGC pathway deficiency on the development of orthostatic intolerance that occurs during re-adaptation to gravity, using the established tail-suspended rodent model to simulate the microgravity conditions.

Aim 2a: To determine the time course of changes in cardiovascular function induced by re-adaptation to gravity in knockout mice (myocardium-specific and vascular smooth muscle – specific) compared with wild type mice. The effects of gene disruption on ECG, arterial blood pressure, heart rate and cardiac Doppler measurements will be recorded immediately and once a day for 3 days following de-suspension in knockout mice versus wild type mice.

Aim 2b: To study the cardiovascular responsiveness in knockout mice versus wild-type mice following de-suspension. Animals will be challenged with vaso- and cardio-active agents. Pharmacological drugs will be administered immediately following de-suspension and once a day for 3 days, as cardiovascular parameters are monitored.

Accomplishments of the project:

1. We used $\text{Lox-}\beta_1$ sGC targeted vector generated previous year to produce gene-targeted mouse ES line. Presently, we on the stage of blastocysts injections with gene-targeted ES clone.
2. In order to gain an advanced knowledge about regulation of sGC expression in human body we continued characterization of β_1 human sGC gene promoter region initiated previous year and identified CCAAT binding factor (CBF) as critically important factor in β_1 sGC expression. The resulting research accomplishments were published in the *Proceedings of the National Academy of Science*.
3. We investigated the role of the heme moiety in the basal state of human sGC and generated the constitutively active heme-deficient sGC enzyme which could be a useful reagent for a gene transfer therapy, amelioration of chronic hypertensive conditions and screening for novel inhibitors and activators of sGC. The resulting research accomplishments were published in the *Proceedings of the National Academy of Science*.

Research plan for the coming year:

To understand the sGC-cGMP regulatory pathway in cardiovascular function we will obtain an animal model with myocardium-specific and smooth muscle-specific disruption of sGC gene expression. In order to achieve this goal: a. β_1 sGC lox knockout mice will be generated utilizing the β_1 murine sGC gene $\text{Lox-}\beta_1$ sGC targeted vector created in our laboratory; b. β_1 sGC-lox mice will be bred with $\alpha\text{MyHC-Cre}$ mice containing a myocardium specific Cre-recombinase, to generate myocardium specific β_1 -sGC-Cre/lox knockout mice and [SM-CreER(T2)(ki)] containing smooth muscle-specific Cre recombinase to generate smooth muscle-specific β_1 -sGC-Cre/lox knockout mice.

Countermeasure Development Plans:

The generation of the sGC mouse knock-out model would help to overcome several problems associated with the study of sGC-mediated physiology. Distinctions between cGMP-dependent and -independent actions of NO, and between the physiological contributions of cGMP produced by the particulate or soluble forms of enzyme, are difficult due to the lack of selective sGC inhibitors. Furthermore, there are at least two sGC isoforms demonstrating very similar pharmacological and functional properties but markedly different expression profiles. Gene-targeted animal models, presently unavailable for sGC, could help to overcome most of these difficulties, allowing discrimination of effects on the level of a single gene. An improved understanding of the mechanisms involved will aid development of new physical and pharmacological measures to counter possible negative effects of changing environmental conditions on the human cardiovascular system. Comparisons between knockout mice and wild type mice under simulated weightlessness conditions will help to identify sGC-dependent pathways necessary for adaptation to microgravity and re-adaptation to gravity. An understanding of the biological and physiological mechanisms of sGC regulation could aid in the development of countermeasures for prevention and/or treatment of negative effects associated with adaptation to microgravity.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Chester A. Ray, Ph.D.
ORGANIZATION:	Pennsylvania State University
PROJECT TITLE:	Effect of Simulated Microgravity on the Vestibulosympathetic Reflex in Humans

Project Executive Summary

Despite the long recognized problem of post-space-flight orthostatic intolerance (OI), the physiological mechanism(s) responsible for this condition remains unresolved. Impaired sympathetic activation is a possible factor for post-space flight OI. One possible mechanism that may be responsible for impaired sympathetic nerve activity after space flight is the vestibulosympathetic reflex. Microgravity has been demonstrated to elicit marked morphological and physiological changes to the vestibular system. Despite this information, no studies to date have examined if the vestibulosympathetic reflex is altered after space flight or its ground-based model for studying autonomic and cardiovascular function, head-down tilt bed rest. The specific aims and hypotheses of this research project are: 1) To determine muscle sympathetic nerve activity (MSNA) responses to head-down neck flexion (HDNF) before and after 1 and 7 days of 6° head-down tilt bed rest (HDBR). HDNF has been used in our laboratory to activate the vestibular system (i.e., otolith organs) in humans and has been shown to increase MSNA. We hypothesize that MSNA responses to HDNF will be attenuated after HDBR and that the attenuation of MSNA will increase as a function of HDBR duration. If this hypothesis is true, this would be the first evidence that the vestibular system may participate in regulating MSNA after HDBR and possibly space flight; and 2) To determine MSNA responses to HDNF during lower-body negative pressure before and after HDBR. We have shown that MSNA is augmented by HDNF during lower-body negative pressure. Thus in healthy adults, the vestibulosympathetic reflex can help defend against orthostatic challenges by increasing MSNA. We hypothesize that the increase in MSNA by HDNF during lower-body negative pressure will be attenuated after HDBR. Therefore, after HDBR the vestibulosympathetic reflex will be impaired and will not be able to help defend against an orthostatic challenge by increasing MSNA. This finding would give credence to the concept that alterations in the vestibulosympathetic reflex may participate importantly in post-space flight OI. These findings should have important implications in understanding the cause of OI following space flight. Moreover, these studies should provide a solid rationale for developing countermeasures involving stimulation of the vestibular system during space flights in order to minimize post-space-flight OI.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Artin A. Shoukas, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Mechanics of Cardiovascular Deconditioning

Project Executive Summary

Changes in cardiac output result from the altered, myocardial contractility or through changes in venous filling pressure via the Frank Starling mechanism. Our laboratory has previously shown the importance of veno regulation by the carotid sinus baroreceptor reflex system on overall circulatory homeostasis, and in particular the regulation of cardiac output. Decreases in SV responses to an orthostatic challenge are the seminal patho-physiologic observation after space flight. In addition there is evidence that there is a significant degree of cardiac hypertrophy after long during space flight. The exact effects of the hypertrophy on ventricular performance is clearly unknown and could significant contribute to the ortho static intolerance after long term space flight. Our proposal aims to test our hypothesis that alterations in venous capacitance function by the carotid sinus baroreceptor reflex system is an important determinant of the cardiac output response seen in astronauts after returning to earth from long term exposure to micro gravity. In addition the physiological effects of cardiac hypertrophy on orothostatic intolerance will be determined. We will use the hind limb unweighted rat model to simulate the patho-physiological effects as they relate ` to cardiovascular deconditioning in micro-gravity. To determine mechanisms of impaired stroke volume responses integrated cardiovascular function (in vivo) and contractile reserve will be tested using miniaturized conductance micro-manometry catheters. The role of cardiac atrophy in cardiovascular deconditioning will be tested using magnetic resonance imaging to noninvasively measure cardiac mass. Since venous capacitance function and arterial resistance determine ventricular preload and after load respectively, mechanisms of impaired contractile responses in both arterial, venous, and pulmonary vascular beds will be studied. Molecular mechanisms of endothelial dependent (eg. nitric oxide), and independent (Ca^{2+} homeostasis and vascular smooth muscle myofilament Ca^{2+} sensitivity) vascular hypo responsiveness to sympathetic stimulation will be studied, using vascular contractility bioassays (in vitro), pressure-dimension analysis both (in vivo and in vitro), and intracellular Ca^{2+} measurement (fluorescence spectrophotometry). We plan to test novel, countermeasures based on mechanisms that impair both cardiac output responses and vascular hypo responsiveness in our rat model. These studies will provide important new data concerning normal capacitance vessel function in compensating for postural blood volume redistribution, test our novel hypothesis regarding the pathogenesis of orthostatic intolerance following micro-gravity exposure, and provide insights into potential countermeasures and therapies to prevent problematic postural hypotension on reentry. Our laboratory currently performs experiments from chronic instrumented animals to the cellular and molecular mechanisms involved in cardiovascular regulation and control.

Key Findings and Summary of Progress

1. We have demonstrated impaired CO responses to an orthostatic challenge in rats following HLU which recovers in ~60hrs.
2. We have demonstrated that after HLU, unstressed venous vascular volume is increased following HLU and can no longer decrease in response to sympathetic stimulation. This

supports our primary hypothesis and may underlie the mechanisms leading to an exaggerated fall in stroke volume seen in astronauts.

3. Using cardiopulmonary bypass studies in which cardiac output is fixed, we have demonstrated that venous and total circulatory capacitance is increased following HLU.
4. We have demonstrated impaired alpha-1-AR and non-alpha mediated responses in large arteries (aorta) of HLU animals. We have also demonstrated that the observed vascular contractile hyporesponsiveness is reversible with time. In addition, alpha-1AR specific abnormalities in mesenteric microvessel responsiveness appear to be present.
5. We have observed a decrease in alpha-1AR specific radioligand binding in aortic vessels from HLU animals.
6. We have demonstrated both an endothelial dependent and endothelial independent component which contributes to vascular hyporesponsiveness following HLU.
7. We have demonstrated an upregulation of the regulatory subunit of myosin light chain phosphatase, a key component of the Rho kinase/Ca⁺ sensitivity mechanisms which regulates vascular contraction. This could contribute to the attenuated agonist induced endothelial independent contractile responses observed in vessel ring preparation.
8. We have established the technique for measurement of phosphorylated myosin light chains as a biochemical determinant of downstream contractile events.
9. We have demonstrated vascular hyporesponsiveness in the large pulmonary arteries of the HLU rats. This vascular hyporesponsiveness is obliterated with nitric oxide synthase inhibition suggesting that increased nitric oxide production may be mediating this impaired contractile response.
10. We have demonstrated an impaired heart rate and blood pressure and contractility response to an orthostatic stimulus (transient bilateral carotid occlusion) in a HLU mouse model using pressure-volume loops.
11. We have developed an external non-invasive mechanical prototype device, in conjunction with the Applied Physics Laboratory of JHU, that peristaltically pumps blood from lower extremities and abdomen towards the heart to maintain stroke volume and cardiac output during an orthostatic challenge. A notice of invention and non-disclosure has been filed with Johns Hopkins University.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Gordon H. Williams, M.D.
ORGANIZATION:	Harvard – Brigham and Women’s Hospital
PROJECT TITLE:	Influence of Gender and Age on Renal and Cardio- Endocrine Responses to Simulated Microgravity

Project Executive Summary

Orthostatic intolerance remains an operational problem following space flight, and has been observed to be more pronounced among women than among men. In addition, there is growing evidence that cardiac dysrhythmias may pose a threat to the health of space travelers.

In our previous studies we observed that subjects on a constant high dietary sodium intake during simulated weightlessness have varying degrees of sodium balance response. Furthermore, the variability in response correlated with orthostatic tolerance and subjects age. Our previous findings also are consistent with an increased basal tone of the RAAS in many subjects during simulated microgravity. These findings are even more intriguing since several subjects demonstrated changes in electrical stability of the myocardium following microgravity. Chronically increased angiotensin II, and particularly aldosterone, levels resulting from bed rest and their effects on myocardial remodeling may be at least in part responsible for these changes. It is not known whether myocardial electrical changes will occur in women or older individuals, or what role the RAAS might play. In the recent Randomized Aldactone Evaluation Study (RALES) trial of spironolactone in patients with congestive heart failure, it was shown that a low dose of spironolactone was protective against sudden cardiac death. Thus, theoretically, if microgravity increases the risk for ventricular dysrhythmias, activation of the RAAS must contribute to this increased risk. Then, a low dose of spironolactone (an aldosterone receptor blocker) may be protective against this phenomenon. Finally, in a previous study we documented that midodrine improved acute orthostatic intolerance following sixteen days of simulated microgravity

We have no data in women and only limited data in older subjects. Thus, the overall goal of this study is to assess in women (a population at increased risk for orthostatic intolerance) and in men over the age of 50 (an age range more consistent to that of astronauts than the <35 year olds involved in our previous studies) the impact of simulated microgravity on volume-regulating systems. A secondary objective is to search for any correlation between changes in these systems and changes in myocardial electrical stability. A final goal is to determine the effect of two potential countermeasures: midodrine in women and low-dose spironolactone in older men. The same methodologies we previously applied to the study of predominantly younger men will be applied in this study. This study is closely related to a companion study “Influence of Gender and Age on Cardiovascular Responses to Simulated Microgravity” by Richard J. Cohen, M.D., Ph.D., Principal Investigator. His study will investigate whether there appear to be any correlative factors between perturbations of the RAAS and effects on myocardial electrical stability.

This work has implications for the treatment and prevention of maladaptive hemodynamic responses experienced by astronauts in flight and on return to Earth. It will increase our understanding of the mechanisms by which weightlessness changes volume and sodium homeostasis, and possibly cardiac electrical stability, thereby, providing entree to develop

appropriate countermeasures. Perhaps most importantly, it will broaden our database to include older individuals and women, two groups who are well represented among our population of current and future space travelers. Finally, the results of these studies may further our understanding of the pathophysiology of alterations in volume homeostatic mechanisms in cardiovascular diseases such as congestive heart failure.

**NSBRI RESEARCH PROGRAM
HUMAN PERFORMANCE FACTORS**

Team Leader:	Czeisler, C. A.	Harvard		
Associate Team Leader:	Brainard, G. C.	Jefferson Medical		
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RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	George C. Brainard, Ph.D.
ORGANIZATION:	Jefferson Medical College of Thomas Jefferson University
PROJECT TITLE:	Optimizing Light Spectrum for Long-Duration Space Flight

Project Executive Summary

Risk factors for the health and safety of astronauts and NASA ground control workers include disturbed circadian rhythms and altered sleep-wake patterns. These physiological changes can result in decrements in alertness, concentration, and performance, all of which threaten the safety of personnel and the objectives of space missions. In studies of astronauts and NASA ground control workers, light treatment has been used as an effective countermeasure to provide entrainment of sleep-wake patterns and other circadian rhythms. It is important to optimize light as a countermeasure for circadian and sleep disruption in space flight missions. For civilians, light treatment is being tested for improving circadian entrainment and enhancing both performance and alertness in shift workers. A Congressional report estimates that 20 million full-time workers in the United States are shift workers and that they have increased health problems including higher risk of cardiovascular disease, gastrointestinal distress, as well as cognitive and emotional problems. The long-term goal of our research is to determine the best wavelengths of light for use as a countermeasure during long duration space flight, as well as for adjusting circadian and sleep disruption in civilians.

Towards achieving this aim, an eight wavelength action spectrum (the relative effectiveness of different wavelengths for eliciting a biological response) has been established to help identify the photoreceptor system for light regulation of melatonin in humans (Brainard et al., 2001b). Ultimately, this action spectrum may be used as a tool for investigating the action spectrum and related photoreceptor system involved in circadian entrainment and phase-shifting. One specific aim of the current research is to extend the action spectrum for light-induced plasma melatonin suppression using monochromatic wavelengths below 440 nm and above 600 nm. These wavelengths are relevant to astronauts who have to adapt to extraterrestrial environments that have spectral characteristics different from those found on Earth. As examples, there is substantially increased short wavelength light below 440 nm outside of the Earth's atmosphere, and Martian skylight has an abundance of long wavelengths above 600 nm. Data from the proposed studies can be used to optimize the lighting environment of astronauts on long term missions. Specifically, these data can be used to 1) improve light treatment as a countermeasure for circadian and sleep-wake disruption in NASA space flight missions, 2) identify the best spectral transmission for space suit visors and the windows used in space vehicles and habitats, and 3) engineer the ideal spectral distribution for illumination of general living quarters during space exploration.

Specific Aims

1) test the hypothesis that wavelengths below 440 nm and above 600 nm are active in regulating melatonin secretion; 2) test the hypothesis that there will be a loss of sensitivity to monochromatic light when the eyes are not pharmacologically dilated during the melatonin suppression trials; and 3) test the hypothesis that there will be a shift in spectral sensitivity of light regulation of melatonin when the eyes are not pharmacologically dilated.

Key Findings

For Specific Aim One, a key finding is that the fluence-response relationship between nocturnal exposure to 420 nm monochromatic light and melatonin suppression is univariant with the dose response with exposures to wavelengths between 440 and 600 nm. Two recent action spectra suggested that a novel vitamin A₁ retinaldehyde-based photopigment may be primarily responsible for melatonin suppression in humans (Brainard et al., 2001b; Thapan et al., 2001). There was poor agreement between these action spectra, however, on the relative sensitivity to monochromatic light at 420-424 nm, allowing for the possibility that the action spectra could be matched to a cryptochrome absorption spectrum. These 420 nm dose-response data suggest that the melatonin action spectrum of Brainard et al. (2001b) fit an absorption spectrum for a novel opsin photopigment which mediates photoreception for the human retinohypothalamic tract. This finding has practical importance to astronauts in long duration space flight since 420 nm irradiance is greatly increased outside the earth's atmosphere (e.g. on the Space Shuttle and International Space Station).

For Specific Aim Two, a key finding is that it takes up to 56 percent more light at 460 nm light for melatonin regulation when the pupils are free to respond to light stimuli. It will be important to further characterize other wavelength responses in freely constricting eyes in order to practically utilize action spectrum data in optimizing light as a countermeasure to circadian disruption during long duration space flight. In almost all cases, astronauts' eyes will be freely reactive during long duration space flight. The wavelength responses in subjects with freely reactive pupils is currently being pursued in Specific Aim Three.

Finally, additional progress that is relevant to all three specific aims involves the completed design and construction of a new Light Emitting Workstation with three times the photon output of earlier generation Light Emitting Workstations. This high output workstation is particularly important to the ongoing work with long wavelength light above 600 nm. Now operational, the light output of this equipment is so powerful that it requires a separate laboratory sequestered from the lower power workstations. The university has provided the needed laboratory space for this equipment along with the necessary waterlines and drains for cooling and a 220 volt electrical supply for power.

Proposed Research Plan for the Coming Year

- 1) Complete manuscript including the final 420 nm fluence-response curve.
- 2) Complete manuscript including two pupillary fluence-response curves.
- 3) Complete study on wavelength response in volunteers with freely reactive pupils.
- 4) Complete 630 nm fluence-response curve.
- 5) Determine feasibility of further work above 630 nm.
- 6) Test fluence-response sensitivity below 420 nm.
- 7) Write final report for first three years of this project.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Charles A. Czeisler, Ph.D., M.D.
ORGANIZATION:	Harvard – Brigham and Women’s Hospital
PROJECT TITLE:	Circadian Entrainment, Sleep-Wake Regulation and Performance During Space Flight

Project Executive Summary

Optimal human performance during space flight requires astronauts to maintain synchrony between the circadian pacemaker, which regulates the timing of sleep, endocrine function, alertness and performance, and the timing of the imposed sleep-wake schedule. Operational demands of space flight necessitate that humans live on day lengths different than the 24h solar day of Earth (Dijk et al., 2001). Due to orbital mechanics, astronauts are commonly scheduled to the near equivalent of a shorter-than-24-hour day length in Earth orbit on space shuttle missions; moreover, they will be scheduled to the 24.65-h solar day of Mars on the planned exploration class mission to Mars.

Over the past ten years, we have successfully implemented a new technology for shuttle crewmembers involving bright light exposure during the pre-launch period to facilitate adaptation of the circadian timing system to the inversions of the sleep-wake schedule often required during dual shift missions (Czeisler et al. 1991). However for long duration space station missions it will be necessary to develop effective and attainable countermeasures that can be used chronically to optimize circadian entrainment during extended duration missions.

The purpose of this 65-day long between subjects randomized study is to test three specific hypotheses aimed at evaluating entrainment of the human circadian pacemaker to longer-than-24-hour days.

Specific aim 1: To test the hypothesis that synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~ 4 percent longer than the pacemakers intrinsic circadian period will be disturbed in men and women;

Specific aim 2: To test the hypothesis that this disturbed circadian synchronization will result in the secretion of the sleep-promoting hormone melatonin during the waking day, disturbed sleep, reduced growth hormone and cortisol secretion, and impaired performance and daytime alertness;

Specific aim 3: To test the hypothesis that two relatively brief (45 minutes) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase, in subjects whose imposed sleep-wake and light-dark schedule is ~ 4 percent longer than their intrinsic circadian period, resulting in improved sleep consolidation, undiminished endogenous growth hormone and cortisol secretion and enhanced daytime alertness and performance as compared to subjects on the same schedule with out the evening bright light exposure.

These hypotheses are based on the results of our preliminary data which indicate that: (a) the period of the human circadian pacemaker after release from entrainment to the 24-hour day is near to but on average slightly longer than-24-hours (Czeisler et al. 1999), (b) the 24.6-h day is outside the range of entrainment of the human circadian pacemaker in the presence of a weak environmental synchronizer (Wright et al., 2001), and (c) intermittent exposure to bright light is a cost effective means of resetting the human circadian pacemaker with respect to power use and astronaut time compared to continuous exposure to light (Rimmer et al., 1999).

During FY02 we completed five experiments. This effort amounts to 325 subject test days in the laboratory during year 2, in addition to the 325 subject days that we conducted in year 1. Originally we proposed to complete 260 subject test days per year and are thus 130 subject test days ahead of schedule. Data collected include: Core body temperature, blood samples (melatonin), Urine samples, Sleep and waking EEG recordings, Subjective sleep quality, Actigraphy, Light intensity, neurobehavioral performance and mood. The successful collection of these data will allow us to test hypotheses 1, 2, and 3 of the project. Data analyses are currently in progress.

The plans for the near future are to continue testing subjects on the Earth and Mars day, analyze data collected, and to test as a countermeasure the ability of brief pulses of bright light to synchronize humans to a dim light-dark cycle for the Earth and Mars day lengths.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

Project Executive Summary

This project is concerned with identifying methods to prevent neurobehavioral and physical deterioration due to inadequate sleep and sleep placed at different times across the twenty-four hour day in astronauts during long-duration manned space flight. The performance capability of astronauts during extended-duration space flight depends heavily on achieving recovery through adequate sleep. Even with appropriate circadian alignment, sleep loss can erode fundamental elements of human performance capability including vigilance, cognitive speed and accuracy, working memory, reaction time, and physiological alertness. When attempting to sleep and perform at an adverse circadian phase, the magnitude and time course of sleep loss and consequent deficits in neurobehavioural functioning are significantly affected. Adequate sleep is essential during manned space flight not only to ensure high levels of safe and effective human performance, but also as a basic regulatory biology critical to healthy human functioning.

There is extensive objective evidence that astronaut sleep is restricted in space flight to averages between 4 hr and 6.5 hr/day. Chronic sleep restriction during manned space flight can occur in response to endogenous disturbances of sleep (motion sickness, stress, circadian rhythms), environmental disruptions of sleep (noise, temperature, light), and curtailment of sleep due to the work demands and other activities that accompany extended space flight operations. The mechanism through which this risk emerges is the development of cumulative homeostatic pressure for sleep across consecutive days of inadequate sleep. Research has shown that the physiological sleepiness and performance deficits can progressively worsen (i.e., accumulate) over consecutive days of sleep restriction, and that sleep limited to levels commonly experienced by astronauts (i.e., 4 - 6hr per night) for as little as 1 week, can result in increased lapses of attention, degradation of response times, deficits in complex problem solving, reduced learning, mood disturbance, disruption of essential neuroendocrine, metabolic, and neuroimmune responses, and in some vulnerable persons, the emergence of uncontrolled sleep attacks.

The prevention of cumulative performance deficits and neuroendocrine disruption from sleep restriction during extended duration space flight involves finding the most effective ways to obtain sleep in order to maintain the high-level cognitive and physical performance functions required for manned space flight. There is currently a critical deficiency in knowledge of the effects of how variations in sleep duration and timing relate to the most efficient return of performance per unit time invested in sleep during long-duration missions, and how the nature of sleep physiology (i.e., sleep stages, sleep electroencephalographic [EEG] power spectral analyses) changes as a function of sleep restriction, the timing of sleep, and performance degradation. The primary aim of this project is to meet these critical deficiencies through utilization of a response surface experimental paradigm. Through testing in a dose-response manner, varying combinations of sleep duration and timing, this project will help establish how to most effectively limit the cumulative adverse effects of chronic sleep restriction in space operations on human performance and physiology.

Although there is evidence that the less sleep obtained, the greater the waking deficits, experiments have found that for acute periods supplementing a reduced anchor sleep period with a nap has the potential to enhance performance, due to the exponential recovery of

neurobehavioral functions relative to sleep duration. During the past 5 years we have been using a response surface experimental approach to systematically determine the chronic (10-day) effects of 18 sleep schedule conditions. There are two experiments in this project. The first experiment involved restricted nocturnal anchor sleep alone and in combination with varying durations of restricted daytime naps on performance, mood, sleep, circadian physiology and hormones. The resulting preliminary response surface maps (RSMs) derived from this dose-response experiment indicate that total sleep time per 24hr is a prime determinant of cumulative neurobehavioral deficits, and that combining a restricted nocturnal anchor sleep with a midday nap can attenuate cumulative deterioration in performance. In order to complete our understanding of how to optimize performance in the face of restricted sleep in space flight, in the second experiment we have reversed the circadian placement of these 18 anchor sleep + nap sleep conditions (i.e., daytime anchor sleep alone and in combination with varying durations of restricted nocturnal naps).

To develop the response surface models, both experiments will require n=90 (total N=180) healthy men and women to undergo a 14-day ground-based laboratory protocol involving random assignment to one of 18 sleep-ration cells. The 18 sleep ration cells utilized in experiment 1 (nocturnal anchor sleep) will be repeated in experiment 2 (diurnal anchor sleep) for a total of 36 sleep ration cells. The sleep-ration assignments involve 4 anchor sleep durations (4.2, 5.2, 6.2, 8.2 hr) and 6 nap sleep durations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 hr) crossed to yield a total of 4 anchor-sleep-only conditions, and 14 anchor + nap sleep conditions, and spanning a dynamic range of cumulative sleep debts (i.e., from 0 to 40 hr in a 10-day period). Subjects undergo a wide range of quasi-continuous neurobehavioral performance tests and continuous physiological monitoring of waking EEG, sleep PSG, behavioral motility, and core body temperature, while living in the laboratory for 14 consecutive days. The laboratory environment is designed to simulate the low light, tight quarters, and lack of social contact with the outside world that will characterize long-duration space flight.

In experiment 1, the data collected from n=91 subjects (i.e. 1,274 laboratory 24-hour protocols) from 1997-2000 investigating restricted nocturnal anchor sleep alone and in combination with varying durations of restricted diurnal naps have been used to create RSMs for different neurobehavioural and sleep variables, including number of psychomotor vigilance task (PVT) lapses, cognitive throughput, subjective sleepiness and sleep efficiency. As data from experiment 2 are obtained, we will (1) establish RSMs for the same variables during simulated night work; and (2) by comparison with the RSMs from experiment 1, determine the role of initial circadian phase of work and sleep on the cumulative rate of impairment from chronic sleep restriction. In addition, these data will be incorporated into our original RSMs, to provide a more complete picture of the effects of restricted sleep schedules, with sleep placed at different circadian phases, on neurobehavioural functioning, sleep physiology and neuroendocrine parameters.

Sleep duration and timing are being covaried in experiment 2 at two sleep-conducive circadian phases: (1) anchor sleep during the daytime and (2) nap sleep during the night. Although scientific evidence strongly supports the view that the less sleep obtained, the greater the likelihood of waking deficits, both laboratory and field studies have demonstrated that a brief preplanned or preemptive nap (0.4 hr to 2.4 hr) may have the potential to sustain optimal performance capability when total sleep time is markedly curtailed. The basis for this disproportionate benefit from a relatively brief nap was recently discovered to be the result of a saturating exponential function, such that the first few hours of sleep net the greatest recovery. Thus, the disproportionate recovery potential of naps may be due to the exponential recovery of neurobehavioral performance functions in relation to sleep duration. This exponential process appears to parallel the time course of EEG slow wave activity (SWA obtained by power spectral analysis) during sleep, which is believed to manifest the physiological homeostatic drive for sleep. The data we have gathered to date in the initial experiment support the conclusion that a

dual sleep period with anchor sleep placed nocturnally and an afternoon nap longer than 1 hr results in normal to high levels of physiologically deep sleep and a reduction in cumulative performance deficits, even when the total time being allocated for sleep in a day is restricted to just over 6 hr.

In experiment 2 we have completed the study of n=48 subjects , for a total of 672 24-hour days in the Sleep and Chronobiology Laboratory. An aggressive data acquisition rate is planned for year 03 that will result in a complete data set by end of year 03. Analysis of the polysomnographic, neurobehavioral and neuroendocrine data is underway on data collected to date, in order to integrate these into our existing response surface models. Due to a reduction in funding from NSBRI for year 02 we did not collect blood samples from May 2002 through October 2002. During this time, n=19 subjects completed the 14-day protocol. Blood sampling was resumed in October 2002 and is ongoing.

We have presented data from the first experiment at the 2002 national sleep conference (annual meeting of the Associated Professional Sleep Societies [APSS], June, 2002) and several other meetings including the recent Bioastronautics Investigators Workshop.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Russell G. Foster, Ph.D.
ORGANIZATION:	Imperial College of Science Technology and Medicine
PROJECT TITLE:	The Role and Characterization of Novel Photoreceptor Mechanisms Regulating Circadian Rhythms, Sleep, Body Temperature and Heart Rate

Project Executive Summary

Varied aspects of physiology and behavior are regulated by gross changes in environmental light. Research leading up to this proposal has shown that in both man and rodents some of these irradiance changes are detected by novel ocular photoreceptors. The photoreceptive mechanisms underlying these, non-rod, non-cone photoreceptors remain uncharacterized. Yet their importance in our daily lives is profound. It is now clear that light exposure can influence alertness and sleep propensity. Furthermore, light regulates the phase of circadian clocks, and thus the timing of rhythmic functions such as digestion, sleep and performance. The central aims of the research outlined in this application are to characterize the molecular mechanism of this unexplored photoreceptor system of the eye, and determine the extent to which these photoreceptors contribute to varied aspects of physiology and behavior. Employing a unique rodless and coneless mouse model (rd/rd cl), and taking advantage of the new opportunities created by a range of post-genomic technologies, including the imminent completion of sequences for both mouse and human genomes, we will address two questions. Question One: What is the role of novel photoreceptors in the regulation of general physiology and behavior? The experiments in this section will determine the extent to which body temperature, heart rate (ECG) and EEG are modulated by non-rod, non-cone ocular photoreceptors. Furthermore, we will define the relationship between novel ocular photoreceptors, light, sleep state and levels of c-fos expression in the ventrolateral preoptic nucleus (VLPO) of the brain. Question two: What are the molecular mechanisms of non-rod, non-cone ocular photoreceptors? Three broad strategies will be undertaken based upon bioinformatics, proteomics, and microarray technology. Our aim will be to identify a set of genes that: a) share sequence similarity with proteins known to be involved in photoreceptor/sensory cell function; b) are expressed in light sensitive cells of the inner retina; c) undergo post-translational modification and/or changes in expression following light exposure. The results from these studies will provide the mechanistic substrate for both targeted drug development aimed at the manipulation of human circadian rhythms, sleep, mood and performance, and the design of new lighting sources that are either highly effective in regulating these novel photoreceptors or leave them largely unstimulated.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Charles A. Fuller, Ph.D.
ORGANIZATION:	University of California, Davis
PROJECT TITLE:	Primate Circadian Rhythms in the Martian Environment

Project Executive Summary

To maintain health and homeostasis, an organism must regulate each of its physiological systems in concert with all of the others and with the external environment. The Circadian Timing System (CTS) has evolved to allow coordination of an organism's physiology and behavior both internally and with the external 24.0 hr terrestrial day. The mammalian CTS is adapted to the lighting environment found on Earth. As we move toward exploration-class space missions, we will be exposing astronauts to non-Earth environments for increasing lengths of time. Changes may include altered gravity and spectral, intensity and day-length differences. This raises the concern of whether or not humans will be able to synchronize to such an alien environment. For example, a Mars-type exploration would entail stays on Mars of one to two years. Compared with the Earth, the Martian environment has a photic spectrum shifted to the red, low illumination level, a periodicity of 24.62 hr, and a 0.38 G gravitational field. The mammalian CTS is most sensitive to light of the blue-green wavelengths and adapted to synchronize to a 24.0 hr day. In addition, light must be relatively bright to affect the CTS of primates, especially humans. Further, altered CTS function including rhythm amplitude and wave form, sensitivity to light, and CTS period, have been reported in both the microgravity environment of space flight and in hyperdynamic fields on the Earth. This program will examine the ability of primates (male and female rhesus monkeys) CTS to cope with the Martian environment. The first three experiments will examine responses to the Martian day, while the last experiment will examine the effects of G on the period of the circadian pacemaker. Experiment 1 will examine the ability of the CTS to synchronize to the Martian photic (spectrum and period) environment. We will examine long-term (4-month) physiological and behavioral responses. Experiment 2 will similarly examine long-term responses to a photic environment composed of a Martian day and Earth light spectrum. Experiment 3 will use the primate model to initiate the development of countermeasures to assure optimum entrainment of the CTS. This experiment will examine the effects of timed bright light pulses on CTS entrainment. Using the forced desynchrony protocol, experiment 4 will examine the effects of 1.0, 1.5 and 2.0 G on the period of the circadian pacemaker. We will develop a G vs. period model to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. This model will be used to develop countermeasure requirements to be tested in experiment 3. Thus, this program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of male and female subjects to altered lighting and gravitational environments.

large scenarios of real situations in order to enhance the prediction capability of Alertness and Performance of crewmembers under different light levels.

- 4) Six different models with different growth factors built on a statistical framework revealed that there exists a predominant 8 to 15 hr rhythm (hemi-circadian) that is independent of the circadian rhythm.

3. The Impact of these Findings:

- 1) The impact of finding 1 above is that we have developed a mathematical method to compare any type of dynamical models on a statistical framework. This approach is very important especially when analyzing the low amplitude core body temperature data. Further, this method is perhaps the only approach available to detect the low amplitude circadian component hidden in the noisy data.
- 2) The impact of finding 2 above is that we could use this framework to compare the available mathematical models of the effects of light on circadian oscillators with respect to their amplitude prediction. It has been shown that two of the recent models are equally accurate in the prediction of phase, but they are quite different in the prediction of amplitude. These observations are very important in the study of sensitivity of the biological clock to light at low amplitude level
- 3) The new version of CPSS can help NASA to design different schedules as well as to predict the performance and alertness of crewmembers prior to a mission.
- 4) There is not much importance given to the study of the hemi-circadian signal observed in the core body temperature data and so far the effect of this signal is ignored in the mathematical modeling. Many investigators consider the hemi-circadian signal as a harmonic of the circadian signal with a strong dependence on the dynamics of the oscillators. However, this finding indicates that the hemi-circadian signal is indeed independent of the circadian signal. This opens a great deal of possibility of investigation to understand the interaction of circadian and hemi-circadian signals.

To effectively use the output of the currently available portable light-measuring device as an input to our Light models we added a new specific aim

Specific Aim 5: To assess the applicability of currently available portable light-measuring/actigraphy devices and light-emitting devices to be used, respectively, as inputs and implementations of the outputs of our Light and Neurobehavioral Performance Models.

4. Proposed Research Plan for the Coming Year:

Specific Aim 1:

- 1) To refine the higher order model's preprocessor using recently completed Intermittent Light studies.
- 2) To further understand the interaction between circadian and hemi-circadian processes to thereby understand the significance of the thermoregulatory process.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Megan E. Jewett, Ph.D.
ORGANIZATION:	Harvard – Brigham and Women’s Hospital
PROJECT TITLE:	Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

Project Executive Summary

1. The Original Specific Aims of our Project:

Specific Aim 1: To further develop and refine our ‘Dynamic Stimulus Processing’ Light Model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting conditions, especially those that occur in space. This will be done using data from four completed studies of the effects on the human circadian system of: *i*) three-cycles of brief bright light pulses; *ii*) three-cycles of extended bright light pulses; *iii*) three-cycles of extended low- and moderate-intensity light pulses; and *iv*) single- and double-cycles of amplitude-suppressing critically-timed extended bright light pulses.

Specific Aim 2: To validate the Light Model refined above in Specific Aim 1 using data from four other completed studies of the effects on the human circadian system of: *v*) single-cycle patterns of brief bright light pulses; *vi*) single-cycle extended bright light pulses; *vii*) single-cycle extended light pulses across a wide range of intensities; and *viii*) sleep-wake/light-dark schedules with a wide range of periods (11-h, 20-h, 23.5-h, 24-h, 24.6-h, 28-h, 42.85-h), different light intensities during wake (1, 8, or 15 lux), and with or without a single exposure to an extended bright light stimulus (in the 11-h condition only).

Specific Aim 3: To incorporate the Light Model refined and validated above in Specific Aims 1 and 2 into our mathematical Neurobehavioral Performance Model, which will then be validated against experimental performance data collected under the wide variety of lighting conditions encompassed in the eight studies described above in Specific Aims 1 and 2.

Specific Aim 4: To develop a user-friendly *Circadian Performance Simulation Software (CPSS)* package that can be used to specify appropriate light schedules as a countermeasure to the poor performance and sleep quality associated with circadian misalignment in space.

2. The Key Findings of the Project:

- 1) An alternate model has been developed with statistical and dynamic properties to analyze the core body temperature data at low amplitude during constant routine conditions.
- 2) A comparative study of previously published dynamical models (Simpler and Higher-Order) based on an alternate model framework revealed salient features in experimental data with respect to Model predictions. Especially at low initial amplitude, it is found that the amplitude recovery of the oscillator is slow, confirming the predictions of the Higher Order model.
- 3) The new version of user-friendly software *Circadian Performance Simulation Software (CPSS)* package has been released. The current version of *CPSS* has been tested with

- 3) To incorporate the higher order pacemaker model into our Light Model to simulate each of the four light studies listed above to determine how well the model is able to predict their results.
- 4) To refine the Light Model as necessary so that it accurately predicts the findings of all four of the studies.

Specific Aim 2:

- 1) To complete the secondary analyses of the non-24-hour day studies so that we have day-by-day phase estimates throughout each of the studies.
- 2) To use the refined Light Model from Specific Aim 1 to simulate each of the four validation studies in order to determine whether the refined model is able to accurately predict their findings.
- 3) To refine the Light Model as necessary until it is able to accurately predict all eight studies listed in Specific Aims 1 and 2.

Specific Aim 3:

- 1) To finish the analysis of the neurobehavioral data from study *vi* in Specific Aim 1 and from study *v* and the remainder of study *viii* in Specific Aim 2.

Specific Aim 4:

- 1) To improve the graphical outputs of the current version of the *Circadian Performance Simulation Software* to allow the user to more easily visualize the schedule they entered and the models' predictions of circadian phase and neurobehavioral performance.

Specific Aim 5:

- 1) To determine the accuracy of the Actiwatch-L's light measurements in sunlight versus room lights.
- 2) To determine the effects of posture on the accuracy of the Actiwatch-L's light measurements.
- 3) To determine the extent to which using Actiwatch-L data for our model inputs improves the accuracy of our model predictions for shiftworkers.
- 4) To determine the actual intensity of light reaching the eye for four different light-emission devices.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Michael Menaker, Ph.D.
ORGANIZATION:	University of Virginia
PROJECT TITLE:	A Model of Circadian Disruption in the Space Environment

Project Executive Summary

In order to discover countermeasures against the deleterious physiological and behavioral consequences of the inevitable disruption of normal circadian rhythmicity produced by the conditions in space, we have first to create a laboratory model of that condition (which for the sake of brevity we are calling “dysphasia”). To be successful such a model must enable us to measure the effects of simulated space conditions on multiple body functions as well as on the temporal relationships of these functions to each other and to the environment. These conditions are fulfilled in large part by our transgenic rat model in which the transcription of the clock gene *Per1* is reported in real time by luciferase. We are able to culture tissues from such rats and to measure the phase of their circadian rhythms in vitro, enabling us to infer their phase relationships in the intact animal. Our aims are first to determine how these phase relationships are disrupted by simulated space conditions, and second to devise counter measures that could in practice be employed by astronauts in space to reinstate normal temporal organization. Of necessity, to be practical, countermeasures must be compatible with the ongoing activities in space vehicles. It is therefore impractical to use the strongest known synchronizing signal, a regular 24-hour light cycle.

We have investigated an alternative synchronizing signal, precisely-timed meals, and have found that its effects are stronger than anticipated and extend deeply into the physiology of the animal. Timed meals set the phase of the circadian rhythms of gene expression in liver, stomach, colon, esophagus, lung, and also the phase of locomotor behavior. Timed meals do not influence the phase of gene expression in the suprachiasmatic nucleus (SCN) or the femoral artery. Our results suggest that timed meals may prove to be a useful partial countermeasure against dysphasia which could be combined with other signals (e.g., melatonin) that preferentially target SCN and/or the cardiovascular system. During the next year we plan to test these hypotheses on transgenic rats made dysphasic by exposure to bright constant light.

The approach outlined above depends on inferences about the behavior of tissues in intact animals based on their behavior in culture. It will be important to confirm these conclusions by direct measurement of the same rhythms in intact animals. This is a technically demanding undertaking, but we are making slow progress by recording luciferase activity with implanted light guides in awake, behaving animals. We will continue to refine this approach.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Lawrence P. Morin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	Circadian and Vestibular System Relationships

Project Executive Summary

The series of studies proposed in this grant, “Circadian and Vestibular System Relationships,” is entirely novel. There is no scientific literature in existence concerning the relationship between the two systems in the grant title. However, at the time the proposal was submitted, a paper had been just published in the *Journal of Comparative Neurology* by Shiroyama and colleagues (1999) showing a direct connection between the medial vestibular nucleus and the ventral lateral geniculate nucleus. However, their data were misinterpreted and the projection is actually to a neighboring nucleus, the intergeniculate leaflet (IGL) of the circadian rhythm system. This opened a lot of possibilities that we developed into a proposal.

Progress with Respect to Specific Aims

Specific Aim One: Connections between the circadian rhythm system and the vestibular system. The first objective has been completed. Using retrograde tracer applied to the IGL, we have shown neurons in the vestibular system project to a nucleus (the IGL) of the circadian rhythm system. We have completed the two planned variations on that anatomical theme. One employed anterograde tracer applied to the medial vestibular nucleus to trace projections to the IGL. The other has utilized a technique novel to this laboratory and has required significant modifications in standard operating procedure. This method uses a transynaptic viral tracer to determine whether two neurons are connected. We have obtained two results of significance: (1) Use of the virus tracing method by itself demonstrates the existence of cells in the vestibular nuclei that have polysynaptic input to the SCN. (2) Use of the virus tracing method in conjunction with a monosynaptic tracer injected into the IGL and the MVe has demonstrated that at least a few of the medial vestibular neurons with multisynaptic projections to the SCN are among those cells that project to the IGL. Furthermore, MVe project to multiple sites which provide afferent input to the IGL, including those which mediate arousal and REM sleep. The results confirm our original expectation that neurons in the medial vestibular nucleus connect to neurons in the IGL that, in turn, project to the circadian clock in the suprachiasmatic nucleus (SCN).

Specific Aim Two: Functional activation of the vestibular and circadian systems by an OKN stimulus. We are assembling the full apparatus and the experiments in this aim are about to start.

Specific Aim Three: Functional activation of the vestibular and circadian systems by a non-locomotor, non-photoc stimulus. Functional implications of a vestibular system activating stimulus are being completed. Thus far 86 animals have been subjected to linear acceleration/ deceleration and rotational stimulation. After the stimulation, the brains were removed, fixed and processed to determine the extent and location of induction of the immediate early gene, *fos*, as indicated by the presence of FOS protein. Preliminarily, the results show a linear relationship between rate of rotation and number of cells in the IGL (among other places) expressing FOS in a lateralized vestibular-stimulus specific manner. Many more animals have been added to the experiment. The data from those brains and target nuclei are in the process of being entered into the computer for analysis in the near future, based on results which emerged from the extended

anatomical study (Specific Aim 1). The ability of the identical vestibular activating stimulus to elicit circadian rhythm phase shifts is being evaluated. The preliminary data indicate that no phase shifts occur; however, there are clear effects on level of post-stimulus motor activity which are differentiated based on the magnitude of vestibular stimulation.

Impact of the Results on the Specific Aims

There are two significant points of impact. *One* concerns the original set of specific aims. Were they realistic and worthy of experimental study? The answer is clearly affirmative. There are no negative changes in the specific aims. We have added rotational stimulation to the originally proposed linear acceleration/deceleration as part of work done for Specific Aim 3. *The second* point of impact concerns the significance of the system being studied with respect to health risks during space flight. The vestibular system influences has a generally pervasive influence on normal behavior. Therefore, knowledge about the routes and mechanisms through which this influence is achieved may be important. In particular, sleep and circadian rhythms may be profoundly disturbed by high level vestibular activation, or might be actually facilitated by low level vestibular activation.

Research Plan for the Next Year

We expect to continue with the approach thus far. With respect to the anatomical work, we are in the final stages of completing a major paper for submission. The text is >95 percent complete and we are selecting, editing and finalizing the figures and verifying all the statements of anatomical "truth." We will finish analysis of FOS protein induction in elements of circadian pathways by vestibular activation. We will complete the study of vestibular activation and circadian rhythm phase shift. We will complete studies of circadian rhythm phase shifts by optokinetic stimulation.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Gianluca Tosini, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Long-Term Exposure to Dim Lighting Desynchronizes the Circadian System of Rats

Project Executive Summary

Many biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations and most of these daily rhythms persist in constant conditions, thus, demonstrating that they are driven by endogenous oscillators. The rhythms that persist in constant conditions with a period close to 24 hours are called circadian rhythms. One the most important aspects of space flight is the absence of geophysical 24-h cycles, which, of course, affects the overall temporal organization of the organisms. In the case of long-duration manned space flight, it is crucial to understand how the whole circadian system would react and behave in such circumstances.

We discovered that exposing rats to constant dim light for 60 days significantly affected the phase-relationship among circadian outputs in the SCN, retina and pineal, demonstrating that in these animals internal desynchronization of the circadian rhythms is occurring. We also observed that the circadian rhythm in arylalkylamine *N*-acetyltransferase (the enzyme that is responsible for the circadian rhythm in melatonin synthesis) was altered in both the retina and in the pineal gland. Our data also indicated that locomotor activity rhythm might be an unsatisfactory marker to assess the circadian status of the whole organism. Internal desynchronization has profound effects on the capability of the organisms to perform (mentally and physically) and to remain healthy. In this research proposal, we have designed a series of experiment aimed to understand the mechanisms that are responsible for the observed desynchronization. We believe that the model we have generated will be useful in to foreseeing and preventing dysfunction of the circadian system that may arise after long periods in the space environment where the normal cycle has been altered.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Fred W. Turek, Ph.D.
ORGANIZATION:	Northwestern University
PROJECT TITLE:	Animal Model for Sleep Loss and Circadian Disruption

Project Executive Summary

The adverse effects associated with imposed disruptions of the normal circadian and sleep-wake cycles are particularly relevant to NASA personnel and their ability to carry out normal duties at a high level of efficiency. Many space travel situations demand that both ground-based and flight personnel engage in duty schedules that can lead to circadian rhythm disruption and sleep loss. The tasks that can be affected involve vigilance, operation and control of vehicles/aircraft, maintenance, preparation and operation of equipment, as well as command and control activities. Night operations are important for successful missions, and there is a clear need to find countermeasures that can alleviate the adverse effects of these activities on human circadian rhythms and sleep as well as on neurobehavioral capabilities and on physical performance.

Despite the high prevalence of chronic partial sleep loss and circadian disruption due to shiftwork in modern society, no animal models have previously been developed to systematically examine the effects of chronic partial sleep and circadian disruption on sleep architecture and performance. The use of a new animal model, as outlined in the original proposal, will lead to new insights into how the circadian and sleep systems are affected by the disruption of their normal phase relationship to one another, and how this temporal disorganization influences neurobehavioral capabilities and motor performance. Information gained using this novel animal model will also be important in the development of effective countermeasures to the adverse effects associated with circadian disruption and sleep loss. These countermeasures could be useful in a number of situations involving NASA personnel, particularly in extended duration space flight missions that will result in challenges to the sleep and circadian system of the flight crew and support teams. This project will also provide important insights in to the interactions between the circadian and sleep/wake systems.

There are three specific aims of the project 1) to determine the effects of 12 hours of imposed wakefulness during both normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measurements 2) to test the hypothesis that treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle, and/or on neurobehavioral and motor performance measurements, 3) to test the hypothesis that access to a wheel (exercise) when in the home cage, will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle, and/or neurobehavioral and motor performance measurements.

Key findings during the past award year suggest that animals forced to be active either during the 12 hour light phase or 12 hour dark phase are successfully chronically sleep deprived by approximately 20-25 percent. The degree of sleep loss seen in these studies is equivalent to a human obtaining approximately 6 hours of sleep per night, which is commonly seen on shuttle missions. It also appears that there are differences in both the amount of sleep lost and recovered each day depending on whether an animal is forced to be active during the light or dark phase. Further analysis of this rich data set is required to elucidate these differences. The onset time of

the circadian rhythm of core body temperature does not appear to be affected by the forced activity protocol under condition of high light levels. However, this may not be the case at lower light levels. Another exciting aspect of the data collected during this unique study is that our results appear to be similar to those seen in data recently published on human subjects exposed to chronic partial sleep loss. This suggests that our unique animal model will be useful in developing new countermeasures that can be applied to humans. It also means that we will be able to successfully model various new or unusual schedules and conditions that humans may be exposed to during space flight or ground operations.

The key findings from the past award year will allow us to further develop our animal model of sleep loss and circadian disruption. During the coming award year we will continue to further analyze the data collected in the past award year. In addition, with a clear baseline of sleep and circadian rhythms established we can determine further the impact of sleep loss on performance and the influence of the proposed countermeasures outlined in specific aim two and three. Initial studies involving a wheel and melatonin administration, will use the imposed period of wakefulness that produces the most disruptive effects on the sleep-wake cycle and/or neurobehavioral and motor performance measurements. We assume that this will be the period of imposed wakefulness scheduled during the light phase (i.e., work during normally inactive period).

**NSBRI RESEARCH PROGRAM
IMMUNOLOGY, INFECTION AND HEMATOLOGY**

Team Leader: Shearer, W. T. Baylor

Associate

**Team Leaders: Butel, J. S. Baylor
Sonnenfeld, G. Morehouse**

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RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Janet S. Butel, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Viral Infections and Mucosal Immunity

Project Executive Summary

Space flight has been found to affect immune responses, and alterations in a normal immune response could have a major impact on the host's ability to control infections. An important question being explored is whether infectious diseases will pose an unacceptable medical risk to the success of long-duration space journeys. All humans are infected for life with latent and persistent viruses, and it is well-known that suppression of the immune system allows latent viruses to reactivate and multiply, which may cause disease in the person undergoing reactivation or in contacts to whom the virus is transmitted. The general hypothesis being addressed is that conditions of long-duration space flight will alter human immune responses, leading to reactivation of latent viruses, increased viral infections and viral disease, and possible development of malignancies, and to altered mucosal immunity, an important host defense against microbial infections. We are focusing on reactivation and shedding of human herpesvirus EBV and human polyomaviruses, agents known to establish persistent infections and to undergo reactivation and cause disease, including cancer, when the host immune system is compromised. Animal models are being used to study radiation effects on host responses to infections, with the suspended mouse model being used for mucosal immunity studies.

There are several ground-based human models that mimic certain aspects of space flight (but not microgravity), including the stress, confinement, isolation, and microbial contamination expected to be encountered during actual space flight. One model we focused on this year involved HIV-infected individuals, a medical condition in which patients suffer immunosuppression due to infection with HIV, the AIDS virus. They are an effective model of medical problems that arise due to damage to the immune system and, by studying patients in various stages of HIV-related disease, different degrees of immune damage can be modeled. First we completed a year-long study of reactivation and shedding patterns of herpesviruses and polyomaviruses in 30 normal, healthy individuals in Houston so that results of virus reactivation studies in ground-based analogs of space flight could be meaningfully interpreted in the context of normal, baseline reactivation patterns. The general approach was that DNAs were extracted from peripheral blood mononuclear cells (PBMCs), urine cell pellets, and saliva and were tested for the presence of viral DNAs by polymerase chain reaction (PCR).

In the healthy adults, JCV viremia over time was 46.7 percent (≥ 1 positive urines over 14 months) with shedding occurring more often in persons ≥ 40 years of age ($p < 0.03$). The urinary excretion of JCV among healthy volunteers was more common in the fall and winter months ($p = 0.05$). No JCV sequences were detected in the blood of any of the volunteers. All 30 subjects in the study possessed EBV-specific IgG antibodies, suggesting prior infection with EBV. Every person shed EBV in saliva at least once during the 14-month period. In contrast to viral shedding in saliva, EBV was detected in PBMCs of only some volunteers (67 percent had detectable EBV in their PBMCs at some time over the course of the study). Levels of EBV varied widely. In the HIV infection study involving 70 HIV-infected individuals and 68 uninfected controls, we found that shedding of both polyomavirus and EBV was elevated in the HIV-positive cohort. Using the CD4 cell count as a surrogate marker for immune status, we found that even modest depressions

in immune function correlated with virus reactivation in both HIV-positive and HIV-negative groups. This emphasizes the importance of understanding the effect of long-duration space flight on the host immune system. Several publications this year resulted from these studies.

A major risk to long-duration space missions is chronic exposure to ionizing radiation, which can damage host cells and immune function. The hypothesis being tested is that space radiation will depress the immune system and lead to reactivation of viruses, increased viral infections, and the development of virus-associated malignancies. We are testing this hypothesis with a mouse model using the murine polyomavirus (PyV) as the mouse equivalent of human polyomaviruses. These studies are being performed in collaboration with Drs. W.T. Shearer and J. Reuben and Dr. Shearer's "Space Flight Immunodeficiency" project and with Dr. D.S. Gridley at Loma Linda University in California. We monitored virus replication in 8 tissues at different time points after infection of normal mice. Length of persistence of virus in different tissues was dependent on the dose of virus inoculated. Once the parameters of virus infection and replication in normal animals were established, experiments were initiated to evaluate the effect of gamma irradiation on host control of viral infection. In preliminary experiments, we have found that gamma irradiation delayed the clearance of virus infection. Follow-up long-term experiments will examine the effect of gamma irradiation on reactivation of latent viral infections and tumor development. In the same experiment spleen cells were harvested and activated *in vitro* with Concanavalin A (Con-A) to assess the proliferation and the production of Th1 and Th2 cytokines by T cells. Preliminary data suggest that soon after irradiation the proliferation and Th1 cytokine production of T cells was suppressed. Moreover, it appeared that the combined effect of irradiation and virus infection further immune suppressed the host. Another goal of the mouse model studies is to define changes in both the mucosal and systemic immune systems under simulated space flight conditions, to determine whether any observed changes would pose significant risks to crew members and to gain basic information necessary for future design and testing of appropriate countermeasures to abrogate detrimental immunologic changes. Lymph node samples from the virus- and radiation-treated mice are being collected for gene expression analysis. These studies also utilize a ground-based anti-orthostatic suspension mouse model. The approach is to catalog global changes in the immune system (cell distributions, cytokine production, gene expression) under simulated space flight conditions and then to determine any additive effects of concomitant virus infection and/or proton irradiation on those patterns. This comprehensive approach will provide new insights into mucosal and systemic host immune functions.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	George E. Fox, Ph.D.
ORGANIZATION:	University of Houston
PROJECT TITLE:	Microorganisms in the Spacecraft Environment

Project Executive Summary

Our work in the past year was focused on four major project areas. We have completed development of a set of 16S rRNA targeted probes that will indicate the presence of major problem and indicator bacteria in flight samples. These probes can be used with similar efficiency under a standard set of operating conditions. The utility of these probes was demonstrated on several samples isolated from various systems including Mars and Moon soil simulants. We also completed the development of a computational algorithm that allows identification of short 16S rRNA subsequences that are highly characteristic of various phylogenetic groupings. In principle, an array of appropriately designed probes based on these signature sequences could be used to determine the genetic affinity (nearest known relatives) in the absence of any prior knowledge of what the problematic organism might be. In practice, the signature sequences themselves tend to be short (15 nucleotides or less) and hence not ideal for use in arrays. We are currently developing an extension to the original algorithm that will allow us to identify very long (30-60 nucleotides) signature fragments whose sequences, despite occasional mismatches, are highly characteristic of various phylogenetic groupings.

An effective set of probes may have utility in a large variety of formats. It is likely that actual implementation in space flight will be driven by mission instrumentation capabilities. Although there is considerable interest in development of array-based instruments other approaches may be preferable, especially for International Space Station applications. We therefore have focused attention on several alternative formats as well. In this regard, several probes for organisms of primary interest have been successfully implemented in a molecular beacon format. Homogenous solution assays of this type would require minimal sample processing and could be readily conducted by astronauts in flight with results signaled by the presence or absence of color changes. It was found that "red-shifted" beacons have minimal contributions from sample autofluorescence. In addition, we demonstrated the potential utility of fluorescent nucleotides such as 2-aminopurine in molecular beacon applications. Efforts were also initiated late in Year 2 to assess the possibility of identifying signature oligonucleotides with mass spectrometry.

Regardless of the assay system ultimately chosen, rapid and simplified systems for sample processing in space will be required. During the past year, we provided further evidence that RNA/DNA purification using compaction agents eliminates the need for preprocessing steps and that the same agents can be used to enhance the adsorption capacity of anion exchangers. Also in the past year we developed further evidence that immobilized metal affinity chromatography (IMAC) which is widely used with proteins is also effective with nucleic acids. Most recently, we have begun to look at novel ways of obtaining rapid final purification of specific RNAs such as 16S rRNA.

There is preliminary evidence that the microgravity environment seen in space affects bacteria in non-obvious ways with such possible outcomes as altered drug resistance or pathogenicity. In order to explore this possibility further, we are examining the response of *E. coli* cells grown in simulated microgravity. In order to do this, we are using modern proteomics technology to

examine the expression levels of each and every gene in *E. coli* when cells are grown in a low shear modeled microgravity (LSMGG) environment. Various kinetic controls have been completed and initial hybridizations with organisms grown under simulated microgravity have been made. These initial studies point to several interesting clusters of genes that appear to be part of a specific response to the LSMMG. In the coming year, we will complete replicates of these initial experiments and examine the results in a multiorganism context.

Overall, Year 2 of the project was very productive. During the past year, four-peer review papers were published, three more are in press, and five additional papers have been submitted. In addition, a book chapter is in press.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Alan M. Gewirtz, M.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Effect of Deep Space Radiation on Human Hematopoietic Stem Cells

Project Executive Summary

Original Specific Aims of Project

Hematopoietic stem cells are the ultimate source of both the blood and immune systems. As such, damage to these cells could have grave immediate, and long-term consequences. Since these cells can be readily removed from the body, manipulated, and stored, they are unique candidates for countermeasures that might obviate, or totally negate, damage incurred to them. Development of such countermeasures is the long term goal of this which is supported by the following specific aims:

1. Investigate the cellular consequences of exposing human hematopoietic stem cells to an environment that simulates the radiation environment of deep space.
2. Examine the molecular consequences of exposing human hematopoietic stem cells to an environment that simulates the radiation environment of deep space.
3. Design potential countermeasures to obviate or negate cellular and molecular damage identified during the course of carrying out Aims 1 and 2 above.

Key Findings

Cell Biology. We irradiated human CD 34+ cells using two types of irradiation: 1) Low linear energy transfer (LET) radiation in the form of the ^{137}Cs - γ rays, and 2) High LET radiation in the form of Fe^{26+} ions generated by the Alternating Gradient Synchrotron (AGS) at the Brookhaven National Laboratory (BNL). We observed that increasing doses of both types of radiation caused increasingly greater decreases in colony number for CFU-GM, BFU-E, CFU-GEMM and CFU-Meg. The decreases observed were more dramatic for Fe^{26+} ions when compared to the ^{137}Cs . This is in accordance with the ‘intensity’ differences in energy that exists between these two kinds of radiation. These results indicate that low doses of both Fe^{26+} and γ -radiation can greatly affect the proliferative capacity of hematopoietic progenitor cells.

We have also generated microarray data on some of these samples, using the Affymetrix Human Focus Array. At 5 GeV, 18 of the 27 genes that decreased significantly also decreased significantly following a 1 GeV exposure. Of interest among these were several RNA processing enzymes, including Dicer, the enzyme responsible for processing dsRNA into RNAi inducing short double strands. This may represent a novel, and unanticipated mechanistic component of cellular response to radiation induced damage.

Radiation Induced DNA Damage and Repair. We found that DNA damage clusters are formed at low radiation doses, and the dose-response relations are straight lines. These results, as well as our low LET radiation data indicate that clusters are formed by one radiation “hit” (including its accompanying track of ions), and thus pose a potential hazard for humans during space travel.

We have also measured repair of double strand breaks, abasic clusters and oxidized base clusters induced by X-rays. These studies showed that in a human monocyte cell line, double strand breaks disappeared rapidly, and few additional double strand breaks (hypothesized to be generated during cluster repair) could be detected. Second, abasic clusters were highly persistent, and significant levels of additional abasic clusters—presumably generated as repair intermediates—were observed. Finally, oxidized base clusters were apparently quite repair-refractory, being detectable for many hours after irradiation.

Finally, we measured repair of clusters induced in human primary hematopoietic cells by Fe ions (1 GeV/amu). In one individual, resealing of double strand breaks remained rapid; some additional de novo DSBs—presumably generated during attempted repair of clusters, thus producing simultaneous scission of both DNA strands—were seen, but were soon undetectable, presumably resealed. Repair of Fe ion-induced abasic clusters was slower than observed for low LET radiation.

Countermeasures. We have investigated two lines of countermeasure development: (1) increasing repair in mammalian cells through increasing levels of DNA repair enzymes, and (2) decreasing DNA damage by growth in increased levels of specific antioxidants.

Regarding (1): We received an isogenic trio of CHO cell lines stably transfected with Fpg protein (glycosylase that carries out the initial step in base excision repair of oxidized purines), and the line containing the vector alone. We found that the CHO cells with increased levels of Fpg protein had the same X-ray survival curves as did the parental line; however, the X-ray mutability of the overproducing cells was strikingly decreased.

Regarding (2): We found that addition of folate or selenium to the medium in which the cells were grown significantly reduced the level of endogenous oxybase clusters, with selenium giving the greatest level of cluster reduction. The mechanism of this reduction is not yet known: is it a chemical effect, reducing the level of clusters induced, or a biological effect, increasing the level of repair of endogenous clusters. It is not also clear whether increased levels of such components in the cellular milieu can also reduce the levels of clusters induced by radiation, specifically those species encountered during travel outside the magnetosphere.

Impact of Findings

The findings outlined above suggest that the possibility of serious, permanent damage to the human hematopoietic stem cell system as a result of exposure to the deep space radiation environment is a real one. The potential for malignant transformation as a result of such damage is also real though at this juncture has not been quantified. The development of effective countermeasures is therefore critical.

Research Plan for the Coming Year

The main focus for the coming year will be to continue experiments characterizing the biological effect of exposure to the ground based-equivalents of cosmic radiation (heavy ions and protons). Drs. Gewirtz and Sutherland propose to combine their respective expertise in stem cell biology and DNA damage/repair to determine these effects. Our studies to date suggest that human hematopoietic stem cells are exquisitely sensitive to even low doses of Fe⁶⁺ particles (see above). Accordingly, all studies proposed on the relative biological impact of HZE particles will be carried out in the dosage range deemed most relevant from our previous studies (<30 cGy). Additional particles (carbon, titanium, protons) have become available at the AGS (Brookhaven National Laboratories). We will carry out dose-response experiments using these new particles,

as in our initial studies, on the well-characterized populations of HSC and HPC and analyze the functional consequences of such exposure using a variety of cell and molecular based assays. We will further identify the molecular lesions responsible for cell damage, which could be of considerable importance in identifying radioprotectants that might ameliorate, or prevent, the damages identified, an issue we also propose to address. Our experiments should detect, and quantify any immediate type damage to various components of the blood cell generating system.

RESEARCH AREA:	Immunology, Infection, and Hematology
PRINCIPAL INVESTIGATOR:	William T. Shearer, M.D., Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Space Flight Immunodeficiency

Project Executive Summary

Original Aims

There are two specific aims to this project concerning Space Flight Immunodeficiency that can be summarized as follows:

A. Specific Aim 1. Using human blood specimens collected in the Australian National

Antarctic Research Expedition (ANARE) winter-over, assess the effects of extreme weather climates, isolation, containment, sleep disturbance, and possible microbial contamination on immune function.

Hypothesis for Specific Aim 1

The ground-based space-equivalent model of the Antarctic winter-over will provide an assessment of some of the conditions of space flight that may have a negative impact upon human immune function.

Objective for Specific Aim 1

Using ANARE plasma samples collected every month from ANARE study subjects stationed in the Antarctic and from control subjects stationed on Macquarie Island, determine whether there are differences in concentrations of cytokines and their soluble receptors, possibly due to the isolation of study subjects.

B. Specific Aim 2. Assess the effects of deep-space radiation and latent viral infection on immune function of experimental animals and determine whether depressed immunity in these animals leads to a state of chronic infection and development of tumors.

Hypothesis for Specific Aim 2

Radiation and virus infection in a murine model will demonstrate a synergistic and permanent depression of immunity, leading to conditions of chronic viral infection and malignancy.

Objective for Specific Aim 2

Using sublethal doses of proton and gamma radiation, reduce immune function in study animals to determine if activation of a latent murine virus induces chronic infection and cancer.

Key Findings

A. Plasma Cytokines and Cytokine Receptor Antagonists in ANARE Subjects

Based in part upon our previous NSBRI-supported human sleep deprivation studies published in 2001, we have extended these studies of plasma cytokines and soluble cytokine receptor antagonists to the space model of the Antarctic winter-over. Australian volunteers were taken by boat from the mainland in October/November 1999 to one of three destinations, two in Antarctica and one on Macquarie Island. The study subjects were those stationed in the two

Antarctic locations (Casey, N=10, Davis, N=11, total N=21), and the control subjects (N=7) were those stationed on Macquarie Island (still had access to the mainland during winter). A period of total isolation for the Antarctic study subjects began in late March/early April, when access to the mainland was prevented by the enlarging ice mass. Monthly plasma specimens were obtained and frozen at -70°C , beginning in January 2000 and ending in September of the same year. After transport of the frozen (dry ice) specimens to Houston, cytokine and cytokine receptor assays were performed with quality-assured ELISA assays. Analysis of the large amount of data is continuing, but several preliminary statements can be made. There appeared to be progressive time-dependent increases in plasma interleukin-12 (IL-12) and interferon-gamma (IFN- γ) levels and time-dependent progressive decreases in plasma levels of interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1RA) in the Antarctic study cohorts, as compared to the Macquarie Island control cohorts. IL-12 and IFN- γ cytokines are important in the activation of cytotoxic T-cells, such as those that might be activated in response to a viral infection – possibly a latent infection that becomes active due to the severe conditions of the Antarctic winter-over. Also, the progressive decrease of IL-10 and IL-1RA levels in the plasma of study subjects in total isolation is a sign of the immune system turning off its anti-inflammatory response, as reflected in the concentrations of IL-10, known to be an anti-inflammatory cytokine and in IL-1RA, a pro-inflammatory receptor antagonist. All of these findings are consistent with the Antarctic volunteers undergoing T-cell activation, possibly due to viral infection.

B. Radiation and Virus Infection in a Murine Model

The co-investigators met in Houston on June 19, 2001 for the team retreat. Dr. Daila Gridley was an invited guest and presented the results of her current work with proton and gamma radiation in mice, demonstrating profound and some lasting deficits of immune function. The following plans were made with regard to collaborative immunology-radiation biology studies. Selected strains of mice (e.g., BALB/c C57 black) will be exposed to proton and gamma ray radiation and subsequently to murine viruses (e.g., gamma 68, polyomavirus) in an attempt to determine the combined effects of space radiation and latent virus infection on the immune function of study animals. This first approach will examine the simultaneous effects of radiation and infection and will then be followed by a sequential approach of infection first and radiation second, the likely scenario for human space travelers to Mars. The dose of radiation that will be utilized initially (3Gy, the estimate of radiation received by astronauts on a Mars Mission) will be that used by Dr. Gridley and her colleagues, who have demonstrated rapid and profound alterations in immune cells and immune responses in murine subjects. Replicate and controlled experiments will be performed by both the Loma Linda University (LLU) site and the Baylor site to insure that the same methods are followed at both sites and that the results of the experiments at Baylor confirm those of LLU. If gamma radiation proves to be equivalent to proton radiation, in terms of effects upon the immune system (e.g., spleen cell T-cell response to non-specific stimuli and specific antigen stimulation; plasma antibody formation to neoantigen; spleen lymphocyte subset distribution), it may be possible to avoid transfer of mice between institutions, as Baylor has a source of gamma radiation. In addition to examination of the effects of radiation and latent virus infection on immune cells and immune responses, study animals will be evaluated for the development of tumors and blood malignancies. This will be carried out with the assistance of Dr. Cory Brayton, a veterinary pathologist at Baylor who has agreed to collaborate on this project.

Because of the complete loss of the vivarium at Baylor College of Medicine, due to Tropical Storm Allison flooding the Texas Medical Center, these small animal studies have had to be postponed. However, the veterinarians at Baylor and LLU have been preparing for these collaborative studies with the submission of documents to the Animal Welfare Committees of

both institutions. The Baylor vivarium is now operating at a ten percent capacity, and soon it will be possible to proceed with the research on Specific Aim 2.

Impact of Findings on Hypothesis, Objectives, and Specific Aims of Original Proposal

A. Plasma Cytokines and Cytokine Receptor Antagonists in ANARE Winter-Over Subjects

The findings of this study strongly support the hypothesis that the immune systems of humans exposed to the long and severe isolation of the Antarctic winter-over become activated by a recurrence of latent virus infection. Therefore, this model of space flight has (to the extent of the present findings) been validated, suggesting that viral infections in space travel will become activated – possibly leading to a state of profound immunodeficiency. These data need further exploration with additional objectives, such as the functional assessment of cellular immunity. Lymphocyte proliferation to specific antigens or the secretion of cytokines in response to specific antigen challenge, or the measurement of T-cell cytotoxicity to virus-infected target cells would be objectives for the next phase of this work. The specific aim would remain the same. It is of considerable interest that these same subjects did not demonstrate defects of B-cell mediated immunity, as assessed with their specific antibody responses to the T-cell-dependent neoantigen, phi-X174 bacteriophage (Shearer, et al., 2001). It is possible that the alterations achieved in T-cell immunity are reflective of T-cell activation only and that immunosuppression did not occur during the one winter-over period. Additional human experiments in this and other models of space flight are warranted.

B. Radiation and Virus Infections in a Murine Model

The difficulties encountered with the Baylor College of Medicine vivarium due to the great flood of 2001 are likely not to be repeated and as more space within the vivarium becomes operational, experiments will get underway. The specific aim and objective remain the same to test the original hypothesis.

Proposed Research Plan for the Coming Year

A. Plasma Cytokines and Cytokine Receptor Antagonists in ANARE Subjects

A complete analysis of the data will be made, and a manuscript will be written for peer-reviewed publication. Plans will be made for additional ANARE experiments in collaboration with Dr. Desmond Lugg. These would include the possibility of performing real time assays of immune function in the Antarctic for the ANARE 2003 expedition. The experience with using frozen specimens delivered from the Antarctic from ANARE 1999 was that too few cells remained viable for analysis of immune function. If the postponed NASA Isolation Capsule Study at the Johnson Space Center in Houston is restarted, it would be possible to perform these cytokine analyses along with the functional assessment of T-cell activation.

B. Radiation and Virus Infections in a Murine Model

See Impact of Findings, B above.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Yufang Shi, D.V.M., Ph.D.
ORGANIZATION:	University of Medicine and Dentistry of New Jersey Robert Woods Johnson Medical School
PROJECT TITLE:	Effects of Antiorthostatic Suspension on the Immune System

Project Executive Summary

Specific Aims

Aim 1: To Investigate the Modulation of Th1 and Th2 Responses in Mice Subjected to Anti-orthostatic Suspension

Aim 2: To Explore the Mechanisms of Anti-orthostatic Suspension-Induced Thymus Involution

Aim 3: To Examine the Role of RANKL in the Communication Between the Immune and Skeletal Systems during Anti-orthostatic Suspension

Key Findings

We have made progress in the following areas:

Hindlimb unloading depletes lymphocytes by distinct mechanisms. Anti-orthostatic suspension by hindlimb unloading (HU) in rodent is a well-accepted ground-based model used to simulate some of the conditions of space flight and reproduce its deleterious effects on the musculoskeletal, cardiovascular and immune systems. The effects of HU on lymphocyte homeostasis in the spleen and thymus of mice were examined. HU was found to drastically deplete various cell populations in the spleen and thymus. These changes are likely to be mediated by apoptosis, since DNA strand breaks indicative of apoptosis were detected by terminal deoxynucleotidyl transferase-mediated nick end-labeling in both splenocytes and thymocytes. Surprisingly, administration of opioid antagonists or interference with the Fas-FasL interaction prevented HU-induced reductions of splenocytes, but not thymocytes. On the other hand, steroid receptor antagonists blocked the reduction in lymphocyte numbers in both spleen and thymus. Therefore, the effects of HU on the homeostasis of splenocytes and thymocytes must be exerted through distinct mechanisms.

Free Radicals Sensitize Splenocytes to Fas-mediated Apoptosis. It has been reported that free radicals are important in stress-induced lymphocyte losses. We have observed that splenocytes from restraint-stressed mice have increased sensitivity to apoptosis in response to JO2 (Fas agonist) antibody *in vitro*, an effect attributed to increased Fas expression by splenocytes. Also, we have recently shown that splenocytes and thymocytes from hindlimb-unloaded mice are more sensitive to dexamethasone-mediated apoptosis *in vitro* and that their increased susceptibility to cell death could be blocked by the anti-oxidant, N-acetyl-cysteine. In light of our discovery of the role of Fas and FasL in stress-induced lymphocyte apoptosis, we hypothesized that free radicals produced during stress conditions promote Fas-induced apoptosis. To test this hypothesis, we exposed splenocytes to H₂O₂ and JO2 antibody, and measured subsequent apoptosis by DNA content analysis using flow cytometry. We found that when either H₂O₂ or JO2 were applied alone, the effect was minimal. However, when splenocyte cultures were treated concurrently with both reagents, there was a significant increase in the number of cells undergoing apoptosis,

indicating that signaling via Fas and the action of oxidative molecules synergistically amplify apoptosis. This finding demonstrates an important link between Fas-mediated apoptosis and sensitivity to free radicals in stress-induced lymphopenia. Further investigation of the molecular mechanisms involved will lead to a better understanding of how stress affects the immune system.

The Effects of Hindlimb Unloading on Lymphocyte Mitogenic Response. The ability of activated lymphocytes to proliferate is essential in promoting an effective immune response. This capacity may be diminished under conditions of space flight. We used our HU model to determine if there is an effect on the proliferative response of lymphocytes. We subjected mice to various durations of HU, then measured the proliferation of splenocytes in response to polyclonal stimulation with anti-CD3 antibody *in vitro* by ³H-thymidine incorporation. HU treatment was found to significantly reduce the proliferative response of splenic T cells. It is interesting to note that the most significant effect occurred after only two days of unloading treatment: a 70 percent decrease compared to control, even though equal cell numbers were cultured. To determine the role of the Fas-FasL interaction in the suppression of cell growth, Fas-Fc was used to neutralize FasL. Adding Fas-Fc to cultures almost completely restored normal proliferation, demonstrating that FasL is essential in the HU-induced suppression of T cell mitogenic responses.

Effect of HU on antigen-specific immune responses *in vivo*. To determine if HU treatment can affect a normal immune response, mice were first primed with ovalbumin (OVA) in complete Freund's adjuvant (CFA), then later challenged with particulate OVA in incomplete adjuvant (IFA) either during, two days before, or two days after HU treatment for 48 hours. The DTH response was measured by changes in footpad thickness 24 hours after challenge. We found that the effect of HU on OVA-induced DTH response varied with the temporal relationship between injection and treatment. The most significant depression of DTH was observed when antigenic challenge occurred during HU treatment. This effect correlated with attenuation of increases in spleen size, as concurrent immune challenge and unloading treatment nearly eliminated splenomegaly resulting from antigenic challenge. Thus, during HU, the capability of the cellular immune system to respond to challenge by a previously-encountered antigen is significantly diminished. This agrees with the recent study reporting that hindlimb-suspended mice are high susceptible to infection with *Klebsiella pneumoniae* (82), in which the Th1 response is critical in resistance. We have started to determine Th2 type humoral immune response by measuring antibody production. Our preliminary data show that OVA-specific IgG2b production is increased in unloaded mice. We are in the process of assaying for other immunoglobulin classes. Though these data are preliminary, they suggest that stress inhibits Th1 type immune responses and boosts Th2 type immunity.

Recovery of Lymphocytes in Spleen and Thymus after Hindlimb Unloading It is well established that stressors of various types cause lymphocyte reduction. Little is known about the recovery process, however. To begin to address this important area, we examined the recovery of lymphocyte numbers following HU treatment. Mice were subjected to HU for four days, then allowed to recover under normal housing conditions. After various periods of recovery, organs were harvested and splenocytes and thymocytes enumerated. We found that overall recovery is quite rapid, with splenocytes recovering sooner than thymocytes. Significant splenocyte recovery was observed after two days, and full recovery by seven days, while full recovery of thymocytes took much longer. This is a rather surprising result, as we expected thymocytes to recover first, then repopulate the peripheral lymphoid organs. It is possible however, that the delayed recovery of thymocytes is due to increased export. Conversely, the faster recovery of splenocytes could be

due to immigration of cells from peripheral sites. Nevertheless, this is an important issue and detailed understanding would contribute to ways of improving the health of astronauts returning from space. Therefore, we continue our experiments to investigate this area.

Radiation Synergizes with Fas-signaling and Hindlimb Unloading to Induce Apoptosis.

Astronauts in space are exposed to various stresses in addition to radiation. While much is known about the effects of stress or radiation, individually, on the immune system, their combined effects have not been thoroughly investigated. Since we have shown that HU-induced lymphocyte apoptosis is probably due to upregulation of Fas expression, we looked first at the effect of radiation on the sensitivity of T cell hybridoma A1.1 cells to apoptosis induced by Fas agonist antibody JO2. While irradiation with 2 Gy gamma rays induced no apoptosis in these cells (A1.1 cells can tolerate up to 8Gy), irradiated cells were much more sensitive to JO2-induced apoptosis. To investigate whether radiation synergizes with stress, we subjected mice to HU for two days and then exposed them to 2 Gy gamma radiation, and continued HU for six more hours. The presence of apoptotic cells in the spleen and thymus was then determined by hypodiploid DNA content. We found that splenocytes and thymocytes from suspension-treated mice were more sensitive to radiation, as they showed a higher percentage of apoptosis after combination treatment compared to either treatment alone. It must be emphasized that mice have a strong phagocytic capacity to remove cells at early stages of apoptosis. Consequently, the apoptotic cells seen in our analyses are only the ones that have overwhelmed the phagocytic capacity; many more must have been cleared and escaped detection. These results demonstrate that radiation synergizes with HU stress to cause high levels of apoptosis in lymphocytes. We intend to further investigate the role of Fas in this process *in vivo* and determine whether high energy particle radiation also synergizes with stress.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Gerald Sonnenfeld, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Suspension, the HPA Axis and Resistance to Infection

Project Executive Summary

The hypothesis being tested is: antiorthostatic (AOH or hindlimb) suspension of mice, a model for some of the effects of space flight on the immune system, results in altered resistance to infection with pathogens. Testing of this hypothesis will provide data to allow development of future studies to determine if space flight affects resistance to infection and if countermeasures can be developed to prevent any detrimental effects.

The specific aims of the study are:

A. To expand the range of infections altered by AOH suspension. We have already shown that resistance to some infections that are not likely to be risks during space flight has been altered by AOH suspension and we now wish to determine if infections that could be a risk during space flight are affected by the suspension model.

B. To determine the mechanism of alteration of resistance to infection induced by AOH suspension. Although previous studies have shown that immune responses are altered by space flight, we now wish to extend these studies to determine the role of neuroendocrine system in regulating infections. This will be carried out using two approaches. The data obtained from experiments using both approaches will be integrated to allow for development of a model for the mechanism(s) of the effects of hindlimb suspension on resistance to infections.

We have completed our study on the effects of hindlimb unloading suspension on resistance to infection with *Klebsiella pneumoniae*. We have found that suspension enhanced mortality of infected mice significantly compared to controls. We also found that suspended mice had impaired ability to clear the *K. pneumoniae*. We also carried out a study to determine the effects of hindlimb suspension on infection of mice with *Psudomonas aeruginosa*. We found that suspension resulted in enhanced mortality of mice infected with *P. aeruginosa*.

We received a contract from a Japanese corporation, the Amino-Up Chemical Company, to test the effects of a nutritional supplement, AHCC, in our model. Our results show that pretreatment with continued treatment of mice throughout the suspension period resulted in protection of hindlimb-suspended mice from infection with *Klebsiella pneumoniae*.

We also continued our studies on the effects of catecholamines on bacterial growth and virulence. We were able to show that growth of anaerobic bacteria was affected by treatment with catecholamines. Treatment of bacteria with the hormone DHEA inhibited bacterial growth. Therefore, we will be exploring use of hormones and nutritional supplements as countermeasures.

We have also begun studies with Dr. Janet Butel of the Immunology, Infection and Hematology Team to determine the effects of catecholamines on viral growth. Additionally, in collaboration with Drs. George Fox and Richard Willson, we have begun studies using array analysis to

determine the proteins that are enhanced when gram negative bacteria have growth enhanced by catecholamines. We are also about to begin studies with Dr. Butel on the effects of catecholamines on growth of viruses.

We have begun studies with Dr. Marcelo Vazquez of the Radiation Team to look at cytokine profiles of mice he currently is exposing to radiation (Protons and Hze) of the head.

We have also prepared a grant to determine the effects of radiation and suspension on the immune system.

Additional experiments have been carried out with Dr. Ann Kennedy of the Radiation Team. We have begun studies with the Bowman-Birk inhibitor, which is a soybean extract that has been shown to have preventative effects on the development of colon cancer. Our studies, which are currently in progress, involve the determination of the effects of the Bowman-Birk inhibitor on the immune system of hindlimb-unloaded mice.

The results of the current research are very much inline with the proposed studies described in the original proposal. We will continue in the next year with the work as outlined in the original proposal.

We will, in the next year, expand our studies with suspension to include gram positive bacteria. We will study the mechanisms involved in the effects of suspension on resistance to infection and the effects of catecholamines on bacterial growth. We will also look at the practicality of expanding development of countermeasures that we have uncovered.

**NSBRI RESEARCH PROGRAM
MUSCLE ALTERATIONS AND ATROPHY**

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RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Parker B. Antin, Ph.D.
ORGANIZATION:	University of Arizona
PROJECT TITLE:	Calpains in Simulated Microgravity-Induced Muscle Atrophy

Project Executive Summary

The overall goal of this proposal is to test the hypothesis that inhibition of calpain activity in skeletal muscles can reduce myofibril degradation and muscle atrophy. Muscle wasting is an important impediment to extended space travel, and studies have shown that muscle size is regulated by the balance between myofibrillar protein synthesis and degradation. Calpain is the major calcium activated protease in animal cells and plays a primary role in regulating the rate of muscle protein accumulation. Considerable evidence suggests that increasing the levels of calpastatin, a protein inhibitor of calpains, enhances muscle protein accumulation. Inhibition of calpain activity, either by increasing calpastatin levels or by expression of dominant negative forms of calpain, may therefore reduce or inhibit muscle atrophy. Research in this proposal will explore these possibilities and has the following specific aims: 1) investigate whether targeted over expression of calpastatin will reduce skeletal muscle atrophy in transgenic mice using the hindlimb unweighting model; and 2) investigate the use of dominant negative forms of calpains to inhibit calpain activity and reduce skeletal muscle protein degradation and atrophy. Studies will use either the muscle creatine kinase promoter or a fully characterized tetracycline inducible system to express calpastatin or mutated calpains in muscles of transgenic mice or in cultured L8 muscle cells. Muscles will be analyzed for changes in overall size, nucleus/cytoplasm ratio, fiber type, total protein accumulation and degradation rates, and accumulation of individual myofibrillar proteins. Information gained is expected to broaden our understanding of muscle growth and may suggest approaches for alleviating muscle atrophy in space and on Earth.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Kenneth M. Baldwin, Ph.D.
ORGANIZATION:	University of California, Irvine
PROJECT TITLE:	Role of Muscle Loading on Mechanisms of Protein Translation and the Impact on Unloading-Induced Atrophy

Project Executive Summary

Specific Aims of the Original Proposal:

The main goals of the proposal are 1) to determine how changes in mechanical loading impact muscle derived IGF-1 gene expression coupled to its signaling pathway activation for modulating regulatory processes that control protein translation efficiency in the context of skeletal muscle growth (hypertrophy) and atrophy; and 2) to systematically develop a rodent resistance training program designed to ameliorate the atrophy process and blunt slow to fast transitions in contractile phenotype. These goals will be accomplished by identifying acute loading paradigms that maintain the target muscle in a positive protein balance state, as defined by the functional status (phosphorylation state and activity) of key proteins known to impact rate-limiting steps in protein translation (initiation and elongations steps) and degradation (disassembly of myofilaments and their degradation via the ubiquitin/proteasome complex) processes. While the focus of the present proposal is aimed at an animal model in order to expedite the attainment of scientific information of a mechanistic nature, we feel that outcomes of such research can be easily translated to studies involving human subjects for subsequent implementation into a viable countermeasure program, e.g., one that is predicated on fundamental scientific principles governing key subcellular processes impacting the protein balance of gravity sensitive skeletal muscle.

In years one and two, we focused on three specific project areas 1) understanding the underlying processes impacting muscle atrophy process in response to complete muscle inactivity, induced by a novel model of unloading called spinal isolation; 2) characterizing the efficacy of different contraction modes for induce skeletal muscle hypertrophy, which could potentially evolve to a paradigm countermeasure for reducing unloading induced atrophy; and 3) assessing the effectiveness of short term isometric training regimens in blunting the rapid phase of unloading induced atrophy which occurs during the first week of unloading. The following are summaries as to what we accomplished in addressing these three important topics.

Atrophy and Protein Deficits in Response to Muscle Inactivity - The goal of this study was to use the model of spinal cord isolation (SI), which blocks nearly all neuromuscular activity while leaving the motoneuron-muscle fiber connections intact, to characterize the cellular processes linked to marked muscle atrophy. Rats randomly assigned to normal control and SI groups were studied at 0, 2, 4, 8, and 15 days after SI surgery. The slow soleus muscle atrophied by ~50 percent, with the greatest degree of loss occurring during the first 8 days. Throughout the SI duration, muscle protein concentration was maintained at the control level, while myofibrillar protein concentration steadily decreased between 4 and 15 days of SI, and this was associated with a 50 percent decrease in myosin heavy chain (MHC) normalized to total protein. Actin relative to the total protein was maintained at the control level. Marked reductions occurred in total RNA and DNA content, and in total MHC and actin mRNA expressed relative to 18S ribosomal RNA. These findings suggest that two key factors contributing to the muscle atrophy

in the SI model are 1) a reduction in ribosomal RNA that is consistent with a reduction in protein translational capacity, and 2) insufficient mRNA substrate for translating key sarcomeric proteins comprising the myofibril fraction, such as MHC and actin. In addition, the marked selective depletion of MHC protein in the muscles of SI rats suggests that this protein is more vulnerable to inactivity than actin protein. This selective MHC loss could be a major contributor for the previously reported loss in the functional integrity of SI muscles. Collectively, these data are consistent with the involvement of pretranslational and translational processes in muscle atrophy due to SI.

Molecular Markers of Atrophy and Protein Deficits in Response to Muscle Inactivity - For this project we examined the expression of several molecular markers of protein balance in response to skeletal muscle atrophy induced by spinal cord isolation (SI, i.e., a complete transection of the spinal cord at both a mid-thoracic and a high sacral level plus complete deafferentation between the two transection sites). This treatment nearly eliminates neuromuscular activity (activation and loading) of the hindlimb muscles while maintaining neuromuscular connectivity. SI was associated with a reduced transcriptional activity (via pre-mRNA analyses) of myosin heavy chain (MHC) and actin. In addition, there was an increased gene expression of enzyme systems impacting protein degradation (calpain 1; plus enzymes associated with polyubiquitination processes) that could further contribute to the protein deficits in the SI muscles via degradative pathways. IGF-I receptor and binding protein-5 (BP-5) mRNA expression was induced throughout the 15-day period of SI, while IGF-I mRNA was induced at eight and 15 days. These responses occurred in the absence of an upregulation of translational regulatory proteins (p70-S6 kinase; 4EBP1) to compensate for the decreased protein translational capacity. These data collectively demonstrate that 1) the molecular changes accompanying SI-induced muscle atrophy are not necessarily the reverse of those occurring during muscle hypertrophy, and 2) the rapid and marked atrophy that defines this model of muscle inactivity is likely the result of multifactorial processes affecting transcription, translation and protein degradation.

Skeletal Muscle Hypertrophy in Response to Isometric, Lengthening and Shortening Training Bouts of Equivalent Duration - Movements generated by muscle contraction generally include periods of muscle shortening and lengthening as well as force development in the absence of external length changes (isometric). However, in the specific case of resistance exercise training, exercises are often intentionally designed to emphasize one of these modes. The purpose of the current study was to objectively evaluate the relative effectiveness of each training mode for inducing compensatory hypertrophy. Using a rat model with electrically stimulated contractions, groups of rats completed 10 training sessions in 20 days. Within each training session the stimulation duty cycle was equal across the three modes. While this protocol provided equivalent durations of duty cycle, the torque integral for the individual contractions varied markedly with training mode such that: lengthening > isometric > shortening. The results indicate that the hypertrophy response did not track the torque integral with mass increases of: isometric 14 percent, shortening 12 percent and lengthening 11 percent. All three modes of training resulted in similar increases in total muscle DNA and RNA. Muscle mass was highly correlated with the 10-session-mean force integral for isometric and shortening but not lengthening actions. The results of this study indicate that relatively pure movement mode exercises result in similar levels of compensatory hypertrophy that do not necessarily track with the total amount of force generated during each contraction.

Isometric-Mode Exercise As a Countermeasure to Unloading Induced Atrophy - Based on the findings, isometric exercise appears to be as effective as both lengthening- and shortening contractions under high loading conditions. Therefore, we have initiated studies to examine the

effectiveness of isometric contractions (Four sets of 10 two-second contractions with 20 second rest intervals between each contraction and five minutes of rest between sets) on its ability to reduce the early-onset of muscle atrophy that is a characteristic feature of the hindlimb suspension model. Preliminary findings clearly show that the mixed fibered medial gastroc (MG) muscle weight /normalized to body mass was significant blunted relative to the contralateral muscle which was unloaded, but not resistance trained. These findings on rats suggest that the simple mode of isometric contraction can be effective in retarding the rapid loss of muscle weight that occurs during the early stages on unloading in which the muscles appear to be the most vulnerable to unloading induced muscle protein degradation.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	P. Bryant Chase, Ph.D.
ORGANIZATION:	Florida State University
PROJECT TITLE:	Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle

Project Executive Summary

The overall goal of this proposal is to produce a muscle cell model (digital cell) that will: explain biomechanical adaptations that occur with alterations in muscle protein isoforms due to changes in activity level; predict bioenergetic changes associated with changes in activity level; and be integrated into computational models of human limb and heart. The essential molecular and subcellular components of the model will be identified and algorithms constructed based on experimental data obtained in a controlled environment. The cell model will be tested against published biomechanical and bioenergetic data obtained under a broad spectrum of environmental conditions. Our muscle cell model will be one of the main building blocks for constructing a model of integrated human function because the cell is the basic unit of physiological organization; the musculoskeletal system is ~80 percent of body mass and thus is a major determinant of energy consumption, as well as being responsible for movement and cardiovascular function. To accomplish our goal of constructing a digital muscle cell, we will: (1) identify contractile protein composition of skeletal and cardiac muscles from high- and low-activity rats; (2) characterize contractile properties (phenotype) of selected muscles containing unique mixtures of protein isoforms, as identified in Aim 1; and (3) develop the “digital” cell biomechanical model.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Alfred L. Goldberg, Ph.D.
ORGANIZATION:	Harvard Medical School
PROJECT TITLE:	The Activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures

Project Executive Summary

Specific Aims

- 1) To clarify the mechanisms that activate the ubiquitin (Ub)-proteasome pathway during muscle atrophy induced by hind-limb suspension and by glucocorticoids which may also contribute to the muscle wasting in astronauts. We have made the surprising finding that one set of ubiquitination enzymes, E2_{14K} and E3 α , which comprise the "N-end rule" pathway, catalyze most of the ubiquitination in atrophying muscles. Therefore, we shall pursue studies to elucidate their special role and how this pathway is activated and contributes to muscle wasting.

- 2) To determine whether pharmacological inhibitors of the ubiquitin-proteasome pathway could be useful as countermeasures to reduce muscle proteolysis and atrophy and to synthesize novel types of inhibitors of this pathway.
 - a) We shall test whether inhibition of the "N-end rule" pathway might be an effective way to prevent atrophy through in vivo studies of transgenic or knockout mice with defects in E3 α or E2_{14K}. Related biochemical studies will attempt to identify more potent inhibitors of E3 α .

 - b) Our other approach toward countermeasure development will be to develop agents that partially inhibit proteasome function in muscle. Because available proteasome inhibitors can block its active sites completely, they are potentially dangerous and can only be used against life-threatening diseases. We recently discovered inhibitory sites in the proteasome by which certain peptides can feedback and retard (but not block) protein breakdown. We hope to synthesize safer types of inhibitors that function by this novel allosteric mechanism to reduce partially muscle protein degradation.

- 3) By using a gene microarray analysis, we hope to identify the spectrum of genes whose transcription rises or falls during muscle atrophy induced by hind-limb suspension or glucocorticoid treatments. Although several changes in transcription have been described in atrophying muscles, in order to fully understand the critical adaptations leading to the loss of mass and functional capacity, it is necessary to obtain a more complete picture of the changes occurring in muscle gene expression. Our initial experiments have already uncovered large increases in seven unidentified mRNAs. Such mRNAs could be useful markers to monitor muscle wasting and the efficacy of countermeasures. Also, identification of their functions could suggest new targets for pharmacological intervention. We shall also carry out a similar analysis of human muscle biopsies taken before and during prolonged bed rest (provided by W. Evans and coworkers) in order to test if insights gained from studies of the rodent models are applicable to atrophying human muscles.

- 4) To identify possible nonpharmacological approaches to reduce protein breakdown, we shall investigate the biochemical adaptations that occur in certain animals to suppress muscle proteolysis and preserve muscle mass. We shall study muscles in two unusual physiological states: in black bears during winter (using biopsies provided by H. Harlow and coworkers) and in rats fed very low protein diets in which muscle protein is preserved despite disuse and decreased caloric intake. In addition, since muscle protein breakdown decreases in rats on protein-deficient diets, we shall test whether in such animals there is less atrophy upon hind-limb suspension.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Marc T. Hamilton, Ph.D.
ORGANIZATION:	University of Missouri
PROJECT TITLE:	Genomics of Human Skeletal Muscle During Bed Rest and Exercise

Project Executive Summary

Reduced use of weight-bearing skeletal muscles during microgravity and sedentary life on Earth causes unhealthy and potentially dangerous consequences. For example, leg muscles atrophy, and also have a profound reduction of lipoprotein lipase activity (an enzyme in the blood vessels of muscles with a protective effect against lipoprotein risk factors for coronary heart disease). It is likely that an unbiased determination of the global expression pattern of the human genome with microarrays will reveal many muscle mRNAs increasing and decreasing, including mRNAs that heretofore have never even been hypothesized to contribute to the “microgravity or sedentary phenotype.” Additionally, large scale genomic studies are likely to begin to reveal clusters of related mRNAs that provide clues as to the sets of genes orchestrating some of the cellular signaling, transcriptional changes, cellular growth, and metabolism. This project will build upon recent experience established from microarray studies of hindlimb suspension, endurance exercise, and muscle fiber type that support the statements described above. The effects of bed rest and one-leg exercise (as a countermeasure to attenuate the effects of inactivity) on the soleus muscle of 6 men and 6 women will be studied. Using state-of-the-art microarray methodologies, this project will measure the expression of ~12,000 full-length sequence verified mRNAs and ~3,000 of the most abundant muscle ESTs. This project is being proposed by a laboratory already using microarrays in the study of muscle physiology, in collaboration with a bioinformatics laboratory, a physical therapy laboratory focused on muscle function, a physician-scientist studying muscle diseases, and a core laboratory for microarray development. This study is likely to discover novel candidate genes and clusters of related genes potentially responsible for the unhealthy responses to reduced muscle use during physical inactivity.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Susan C. Kandarian, Ph.D.
ORGANIZATION:	Boston University
PROJECT TITLE:	Gene Expression Profiling of Unloaded Skeletal Muscle

Project Executive Summary

Original aims of the project

The overall goal of this project is to elucidate signaling mechanisms that mediate the adaptation of mammalian skeletal muscle to mechanical unloading. In identifying cellular mechanisms we will be in a better position to develop effective countermeasures to combat the deleterious changes in muscle function. Global gene expression profiling will be a major tool used to address this goal. The specific aims were:

To conduct global gene expression analysis in mechanically unloaded mammalian skeletal muscle. (Completed 2002) - Affymetrix RatU34A GeneChips were used to probe mRNA expression in rat soleus muscle after 1, 4, 7 and 14 days of hindlimb unloading.

To identify candidate factors and pathways involved in the regulation of unloading induced muscle atrophy. (Completed 2002) - Data analysis software was used to identify genes that are sensitive to unloading, initially by plotting expression of genes in known functional categories and pathways over time. Clustering algorithms were then used to elucidate sets of genes, with known or unknown functions, that are co-regulated based on temporal expression patterns. These approaches provided insight into possible gene associations and candidate players in the pathways that regulate the atrophy process, and thereby proposing them for further study.

Quantitative analysis of candidate factors and pathways involved in regulating unloading induced atrophy. (In Progress) - Interesting candidate factors or pathways identified in Aim 2 are being reconfirmed using more quantitative and focused methods. These include northern analysis and RT-PCR, western assays to quantitate protein levels, activity assays for kinases or phosphatases, immunohistochemistry to localize the cell type of protein expression, and in vivo overexpression experiments of these candidate genes.

Identification of conserved regulatory elements in co-regulated genes. (Changed focus of this aim.) - Local sequence alignment algorithms was proposed be used to identify regulatory sequences conserved among genes that are co-regulated.

Key findings of the project

The results from the initial phases of the project have recently been published in their entirety (Stevenson 2003). In brief, expression of 309 known genes was significantly changed by at least 2-fold (212 upregulated, 97 downregulated). *K*-means clustering was used to divide these genes into co-regulated clusters based on the similarity of temporal expression patterns. This allowed the development of a timeline of the atrophy process with respect to the behavior of genes in a broad array of functional categories. Regulatory genes were often upregulated early, in either a transient or sustained manner, but they also populated clusters with later patterns of activation, suggesting different phases of molecular adaptations. Other early events were the activation of ubiquitination genes and downregulation of protein chaperones. In comparison, clusters

representing slightly delayed activation patterns included genes involved in fast contraction, glycolysis, translational inhibition, oxidative stress, protein degradation, and amino acid catabolism. Downregulated genes exhibited fewer unique expression patterns and included structural and regulatory genes of the extracellular matrix and cytoskeleton, and genes that define a slow-oxidative phenotype. Other novel findings include the tight co-activation of proteasome subunit and ubiquitination genes, differential regulation of serine proteases and serine protease inhibitors, and the identification of transcriptional, signaling, growth and cell cycle genes that probably play a role in the atrophy process. The present work has uncovered temporal patterns of gene expression that highlight the molecular processes that underlie muscle atrophy and provide new avenues for study.

Nedd4 project: Results from several laboratories have shown that the ubiquitin-proteasome system is responsible for the majority of muscle protein loss that occurs with disuse. Ubiquitin-protein ligases (E3s) are responsible for the targeting of specific proteins for degradation by the proteasome. Our microarray data reconfirm that Atrogin1 and MuRF1 are upregulated during unloading, but we have also identified another upregulated ubiquitin-protein ligase not previously characterized with respect to muscle atrophy called Nedd4. Nedd4 has a pattern of activation very similar to that of Atrogin1/MAFbx. We have confirmed this pattern of activation at the mRNA and protein level (see section II). Nedd4 is known to ubiquitinate membrane proteins but its role in muscle protein turnover has not yet been defined. We are in the process investigating the role of Nedd4 in muscle using the tools described.

Impact of findings on objectives

The data summarized above suggest several pathways that are at work during muscle atrophy. These include the activation of proteolytic systems, the inhibition of translation, oxidative stress, extensive remodeling of the extracellular matrix and cytoskeleton, and the activation of several signaling pathways including the JAK-STAT pathway, notch signaling, cytokine signaling, and myogenic signaling. The results also indicate that there are several different temporal switches of regulatory genes that are activated during atrophy. That is, not all transcription factors and signaling factors are up or down regulated early, and not all of them have the same pattern. Some are transient and some are sustained. Also, one of the original aims of this project was to identify specific genes that may be involved in the regulation of muscle protein loss during atrophy. We have carried out some focused study on the Nedd4 gene and this work is providing us with a better understanding of this ubiquitin ligase in proteolysis during muscle atrophy.

The next step towards studying the function of these putative regulatory genes is to overexpress or inhibit their actions in muscle cell culture or in vivo. In order to study the role of the candidate genes we have identified from our microarray analysis of atrophying skeletal muscle, we are using conditional expression systems or transduction of candidate genes post-differentiation in cell culture and in vivo. This will allow us to limit the expression of the transgenes to differentiated myotubes or adult skeletal muscle. The approaches we are using for the overexpression or inhibition of candidate genes are: a doxycycline induced conditional expression system and when higher levels of transfection are necessary and adenoviral expression system. For in vivo experiments we are using direct plasmid injection with electroporation or injection of an adenoviral vector, each encoding one of the several genes we will study in either wild-type, constitutively active, or dominant-negative form. We will then test the effects of the overexpression or inhibition of these genes in whole muscle or in muscle cell culture using standard biochemical and morphological assays.

For the last aim, we have changed the direction slightly. Instead of examining the regulatory regions of genes whose expression was similarly regulated, we are examining the regulatory regions of genes changed in response to unloading for a specific consensus sequence. This sequence is the binding sequence for the transcription factor, NF- κ B. The reason for doing this is that we have shown that NF- κ B transcriptional activity is necessary for muscle unloading atrophy. In order to identify the target genes of NF- κ B during atrophy, a first step is to identify all the genes that were upregulated that have a NF- κ B binding site in their regulatory region. This is being done using publicly available computer programs, "Promoser" and "Patser" (for "pattern search"). We have identified 31 genes that are potential NF- κ B targets using the list of changed genes from the GeneChip dataset. Then fragments of the regulatory regions from these genes can be cloned into a reporter plasmid, injected into muscle, and tested as to whether the reporter is upregulated in response to unloading. If it is, then the NF- κ B site(s) will be mutated and the experiment repeated. If the mutated plasmid does not have unloading sensitivity, then this gene would be a direct target of NF- κ B during muscle atrophy, and could be studied further.

Proposed research for the coming year

Development of expression systems to test candidate regulatory genes: To determine whether the overexpression of candidate genes can regulate markers of atrophy in muscle cell culture. First we will test if candidate genes are sufficient to induce myotube atrophy in cell culture. Initial genes to be studied include those that were activated early with unloading, had sustained activation, and are involved with protein degradation. These genes include cathepsins C, D, and L, the ubiquitin ligases Nedd4 and atrogin-1, and calpastatin. As atrophy also involves decreased protein synthesis, we will also study IGFBP5, a gene involved in the negative growth control of IGF-1. We are using doxycycline inducible and adenoviral expression systems to limit gene overexpression to differentiated myotubes. As endpoint markers of atrophy we will measure cell diameter and DNA/protein ratio, but we will also measure changes that represent intermediate steps in the atrophy process. These include the phosphorylation state of proteins involved in translation, the activity of proteolytic systems, and protein synthetic and degradation rates. In addition, co-expression strategies will be used to test whether multiple genes more effectively induce markers of atrophy. Where possible we will also test whether the inhibition of candidate genes can attenuate atrophy in a myotube atrophy model, thus testing whether the candidate is necessary for atrophy in cell culture.

Future Years

To determine whether the overexpression or the inhibition of candidate genes can regulate atrophy in vivo. To test if the genes mentioned above are sufficient to induce markers of atrophy in vivo, we will overexpress candidate genes in rat soleus muscle using plasmid electrotransfer, adenovirus or adeno-associated virus infection. To test if a candidate gene is necessary for unloading induced atrophy, we will inhibit its expression by injecting a dominant negative or an anti-sense version of the candidate gene contained in plasmid or viral expression vectors. We will then measure if unloading atrophy can be attenuated in fibers containing the transgene. Uptake by individual muscle fibers will be tracked using epitope-tagged cDNAs or EGFP fusion proteins in order to colocalize overexpression with the effects on fiber size. Knockout mice will also be used to evaluate the ability of candidate genes to attenuate unloading muscle atrophy.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Martin J. Kushmerick, M.D., Ph.D.
ORGANIZATION:	University of Washington
PROJECT TITLE:	Integrating Human Muscle Energetics and Mechanics

Project Executive Summary

Background

Human muscle performance depends on a number of factors beyond the mechanisms in neural activation and control. In fact, the experienced and trained nervous system depends on stable biomechanical properties of the muscle for skilled and reliable limb performance. The biomechanical properties depend on the size of the muscle (muscle quantity) and the phenotype of the muscle cells (muscle quality). Most of the other projects in the muscle team are concerned with the regulation of muscle mass (risk of wasting and atrophy).

This project uniquely analyzes by experiment and modeling the interplay between biomechanical properties and energy metabolism. That this is important is obvious from the facts and extensive literature on the sustainability of muscle performance and ease of fatigue. Decreased performance and fatigue is determined by an integration of muscle properties, mass of muscle and biomechanical demand. The first part of this project analyzes and quantifies the internal structures of human limb muscle, with the goal of translating external torque and length changes produced across a limb by a given muscle into the actual forces and lengths of the muscle cells experience when organized as fascicles. This work enables a cellular and mechanistic analysis well beyond what is currently done and described in the literature for human muscle.

Biomechanical power output must be matched quantitatively by biomechanical power input within the cells for sustained activity beyond a few tens of seconds. The second arm of this project applies quantitative measures of the components of energy balance (ATPases, glycolysis and oxidative phosphorylation) to measure the energetic properties of selected muscles and how these vary among individuals. These measurements are then analyzed in the third arm of this project by a quantitative model to obtain metabolic fluxes and in combination with mechanical data to obtain quantitative analyses of economy, efficiency or doing work and related parameters.

Goals

The overall goal of this project is to build up a series of measurements and models of:

- Intracellular energy and metabolic fluxes (ATP supply and demand);
- Mechanics during exercise (ATP demand); and
- Blood flow (ATP supply).

The goal will make possible an integration of the mechanisms involved and a validation of models describing these processes.

Aim One: Measure the economy and efficiency of human muscle contraction and sustainable power output.

Hypotheses tested:

1. The balance between ATP supply and ATP demands account quantitatively for the difference in sustained performance (duty cycle) in various muscles and individuals.

2. Working contractions add a substantially larger, myofibrillar cost above isometric twitches in which only ion transport activation costs dominate.
3. Muscles differ in economy and efficiency: mechanically slower muscles have higher economy and thermodynamic efficiency for converting ATP to external work than fast muscles, but lower power.

Aim Two: Integrate the component mechanisms of energy supply and demand into models to make predictions of energy costs and balance in new experimental conditions.

Hypotheses tested:

1. Simple models of the components of energy balance as developed in aim one are necessary and sufficient to account for the major energetic properties of human muscle.
2. The models establish a cellular basis for defining isometric economy and working efficiency.
3. Variations in properties among muscles and individuals define the normal distribution of properties so this distribution can be used to define probabilistic responses of the system.
4. When new mechanistic components at the molecular and cellular level are added, they can be tested for their effects on the system operation.

Relevance to Risk Reduction and Countermeasure Development

Even in the presence of significant atrophy muscle may be capable of sufficient and sustainable power output provided the muscle is operating over an appropriate portion of the force-velocity and power-velocity curve and provided that there is sufficient steady state and dynamic metabolic power. While not minimizing the importance of adequate muscle mass and the deleterious effects of muscle atrophy, we focus on the equal or more important aspect of the muscle functional properties in intact humans by entirely non-invasive methods, some of which can be conducted in the International Space Station and on long-duration expeditions.

Current risk assessment is based entirely on evaluating the consequences of decreased muscle mass. The information provided by this project enables an evaluation of the consequences (both positive and negative) of altered muscle performance. The modeling enables a forward analysis of altered exercise strategies to accommodate possibly the same motor tasks. It also enables a prediction of the biomechanical responses to the muscle phenotype that would be produced by countermeasures developed by molecular and pharmaceutical procedures being investigated in other projects of the team and by exercise strategies being developed by other teams and by JSC and other NASA professionals. It is likely that the bioenergetic/biomechanical analyses would be useful in design of space suits because of the inevitable addition of mass, friction and viscosity of the suit to the overall limb function.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Michael B. Reid, Ph.D.
ORGANIZATION:	University of Kentucky Medical Center
PROJECT TITLE:	Redox Modulation of Muscle Function in Microgravity

Project Executive Summary

Exercise-induced fatigue and muscle atrophy are mediated in part by reactive oxygen species (ROS), a stimulus that may be exaggerated by radiation during spaceflight. The current project is assessing the roles of ROS signaling and radiation on muscle fatigue and atrophy and is testing antioxidants as possible countermeasures. Progress was hindered by severe damage to our institution by Tropical Storm Allison in June, 2001. These losses were resolved and we have made rapid progress on the project over the last year as outlined below:

Aim 1. To determine if oxidative stress contributes to muscle fatigue during handgrip exercise. Fatigue of hand and forearm muscles may limit crew performance during extravehicular activity (EVA). N-acetylcysteine (NAC) is an antioxidant that inhibits muscle fatigue in humans. We have recently completed experiments testing the capacity of NAC to inhibit muscle fatigue and oxidative stress in humans during handgrip exercise. Working with Dr. Jeff Jones, Flight Surgeon at NASA Johnson Space Center, we used equipment and test procedures designed for use on the International Space Station. Results of the study show the feasibility of this approach. NAC abolished glutathione oxidation blood draining the affected muscle groups and increased handgrip endurance by 30 percent relative to untreated trials. This aim has been completed and a manuscript is being prepared. Follow-up studies are being planned that will test the importance of these findings in a more operationally-relevant setting.

Aim 2. To determine whether ionizing radiation accelerates ROS production and fatigue in skeletal muscle. We postulated that proton radiation absorbed during EVA would increase tissue ROS levels and accelerate muscle fatigue. We were testing this postulate in collaboration with Dr. Carlos Gonzalez, Director of the cyclotron at the University of Texas Medical School, when Tropical Storm Allison destroyed the cyclotron facility in 2001. The facility has not been rebuilt. Resources intended for these experiments have been redirected to studies of mechanisms regulating muscle atrophy and to tests of potential countermeasures (Aim 3).

Aim 3. To evaluate oxidative stress as a mediator of muscle weakness caused by gravitational unloading. Muscle atrophy and contractile dysfunction cause weakness after prolonged spaceflight. We are evaluating oxidative stress as a cause of these changes in mouse soleus during 12-days of hindlimb unloading and are using cell culture techniques to evaluate mechanism. Our results show that: 1.) unloading increases oxidant activity within soleus muscle fibers; 2.) contractile dysfunction is blunted by administration of some antioxidants (NAC, allopurinol) but not others (curcumin, vitamin E); 3.) a novel ubiquitin conjugating enzyme, UbcH2/E2_{20k}, is highly expressed in skeletal muscle, is upregulated by ROS exposure, and mediates ubiquitin conjugation to muscle proteins; 4.) hydrogen peroxide upregulates expression of atrogin1/MAFbx, a key ubiquitin ligase that regulates muscle atrophy; 5.) this signal is transduced by p38 MAP kinase; and 6.) p38 inhibition blocks atrogin1/MAFbx upregulation and the associated rise in ubiquitin conjugating activity. In Year 3, we will test the roles of these transcriptional mechanisms in atrophy of unloaded muscle and will continue to evaluate potential countermeasures.

Aim 4. To determine if radiation stimulates atrophic signaling in muscle We postulated that radiation-derived ROS might stimulate catabolic signaling and planned to measure activity of redox-sensitive pathways in muscle after proton irradiation. Destruction of the Medical Center cyclotron has prevented these experiments. Project resources have been redirected to studies of cellular mechanism and putative countermeasures (Aim 3).

RESEARCH AREA:	Muscle Alterations & Atrophy
PRINCIPAL INVESTIGATOR:	Shantanu Sinha, Ph.D.
ORGANIZATION:	University of California, Los Angeles
PROJECT TITLE:	In-Vivo Stress-Strain Dynamics in Human Muscle

Project Executive Summary

Muscle atrophy is a complication of prolonged exposure to microgravity and likely involves an alteration in the strain properties of the muscle-tendon unit. Such alterations in biomechanical properties are likely to predispose muscle to strain injury as well as create errors in motor function, both in the microgravity environment and upon return to Earth-based activities. Quantifying the magnitude and distribution of the stress-strain properties of muscle during both the atrophic and recovering state is a specific aim of this project. Preliminary evidence shows that the strain distribution within muscle is highly heterogeneous but closely linked to the anatomical architecture of the muscle. Reduced levels of mechanical load likely plays a significant role in the development of muscle atrophy through alteration of the strain characteristics of muscle, (magnitude and the strain distribution). In our experimental design, a group of subjects (n=12) will undergo unilateral lower limb suspension (ULLS) for 6 weeks to induce muscle atrophy of the triceps surae muscle complex (TSMC) in one extremity. Strain magnitude and distribution within the muscle will be measured by velocity encoded cine-phase contrast MRI during an *in vivo* isometric contraction of the TSMC with maximal effort. Static muscle volumes of individual muscles of the TSMC, peak muscle velocity, and torque at that point of time will also be quantified. A custom designed force transducer apparatus will measure torque. The scanning and testing sessions for the ULLS group will be at 2 weeks and 1 day prior to suspension, last day of a 6-week suspension period and 2, 4, and 6 weeks post suspension. The muscle stress-strain dynamics will demonstrate the regions of muscle most affected by visualization and quantification of atrophy and the corresponding susceptibility to strain injury. Development of the MRI technology used in this study should be useful in future studies to test the efficacy of a wide range of exercise countermeasures by providing an objective measure of changes in stress-strain properties and recovery of these parameters following muscle atrophy.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Robert W. Wiseman, Ph.D.
ORGANIZATION:	Michigan State University
PROJECT TITLE:	Ca⁺² Homeostatis and Muscle Phenotype: Role of Cellular Energetics

Project Executive Summary

Exposure of skeletal muscle to space flight results in a significant loss of mass and a shift in the phenotype from slow to fast muscle isoforms. To a limited extent, astronauts are able to ameliorate this remodeling of muscle tissue through exercise. If the mechanistic link between physiologic function and phenotype were better understood, design of countermeasures using combinations of exercise protocols and pharmaceuticals could be employed to increase the efficacy of training while on space missions. We propose that altered physiologic function signals the initiation of the remodeling process through Ca⁺² sensitive transcription factors (CSTFs) which are activated through changes in two homeostatic processes; mitochondrial ATP synthesis and sarcoplasmic reticulum (SR) ATPase Ca⁺² handling. It is our assertion that alterations in phenotype in response to changes in load bearing or any other metabolic stress involves processing information from the physiology in the form of feedback from these two homeostatic processes. We use an integrative approach to study this problem in isolated superfused skeletal muscles using a combination of non-invasive techniques (³¹P-NMR and fluorescence spectroscopy and mechanics) and molecular techniques. In the first Aim we determine the sensitivity of cytosolic Ca⁺² handling to metabolic loads induced by electrical pacing and metabolic inhibitors. In the second aim we test the response of CSTFs to alterations in Ca⁺² homeostasis using ionophores, SR ATPase inhibitors as well as the metabolic stresses we develop in Aim 1. We believe once the mechanistic link is established that we may be able to design countermeasures to mask the loss of mechanical loading by direct manipulation of cytosolic Ca⁺² and more effectively stave off the changes occurring in limb musculature.

**NSBRI RESEARCH PROGRAM
NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS**

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RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Gary Aston-Jones, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Stress, Performance and Locus Coeruleus

Project Executive Summary

Original Aims

The original aims of this project were: 1. Analyze the activity of brain stem noradrenergic locus coeruleus (LC) neurons during a continuous performance task, 2. Determine the effects of acute and repeated stress on changes in LC function and performance, and 3. Identify pharmacological countermeasures to mitigate stress effects on LC activity and attentional function.

Key Findings

1. *Development of a continuous performance task for the rat.* We developed a target detection continuous performance task that rats can learn rapidly. This task mimics many of the attributes of the target detection task in our previous studies in monkeys in which LC activity appears to play a major role. Rats initiate each trial by pressing one lever, and then must discriminate between two signal lights to determine if the one illuminated is a target or non-target. If the target signal light is illuminated the rat must press a second lever to obtain food reward. If the nontarget is illuminated he must withhold responding with no reward and await the next trial. Targets occur randomly on 20 percent of the trials. This task will be the means by which we measure performance abilities and changes therein induced by stress and pharmacologic treatments.

2. *Effects of stress on performance in the target detection task.* To date we have tested only acute noise stress on performance of this task. Results indicate that white noise during task performance at 90 db significantly increased responding to the non-target stimulus (false alarm (FA) errors) in this task. Interestingly, this effect habituated rapidly, so that subsequent administration of 105 db did not influence performance. Chronic stress (planned for this year) may be needed to see continued performance deficits. The alpha2 adrenoceptor agonist clonidine (which decreases LC-NE neurotransmission) at 8 mg/kg reduced the FA error rate seen with 90 db noise stress. Higher doses of clonidine (25 mg/kg) produced sedation. These preliminary experiments require confirmation with additional studies, but they suggest that the NE system may be involved in stress effects on performance in this task.

3. *Effects of idazoxan on performance in the target detection task.* The alpha2 adrenoceptor antagonist idazoxan increases firing of LC neurons and release of NE from LC terminals. Our view of LC's role in performance predicts that this agent should worsen performance on this task, with increased FA errors (as observed in monkey LC neurons during periods of high tonic LC activity). Systemic idazoxan had no effect on two rats that were performing marginally in the task (i.e. a 30 percent false alarm rate). However, this compound markedly increased false alarms in both of the rats that were performing exceptionally well and had low baseline false alarm rates in the absence of the drug. Although preliminary, these results are consistent with the view that moderate levels of tonic LC activity are critical for maintaining focused attentiveness to task stimuli and performing optimally, and that behavioral performance declines when tonic LC firing rates are increased. We speculate that the lack of an effect of idazoxan in rats with marginal baseline performance reflects the inverted U relationship described by the PI for the relationship

between LC activity and performance on such a task. Thus, in these rats the poor performance pre-drug may have been due to a high level of baseline tonic LC activity, placing them at the right of the inverted U relationship. This pre-existing heightened LC activity could have created a ceiling effect that prevented idazoxan from further increasing LC firing rates and disrupting responses.

4. Role of the LC in circadian regulation of sleep and waking. We expanded our program to include analysis of the role of LC in effects of sleep deprivation on performance. We took this step because sleep deprivation is one of the largest stresses affecting the astronaut, and there are well-established effects of sleep deprivation on performance. For this, considerable technological development has occurred. We implemented a telemetry system for recording EEG, EMG, body temperature and locomotor activity in freely moving, untethered rats. This system produces robust sleep measures over long periods of time. We have also developed a mechanism for producing sleep deprivation, consisting of a slowly rotating wheel that the rat is within. This device allows access to food and water and also contains levers and stimuli to allow task performance during the sleep deprivation period. We will use this system to deprive rats of sleep at different times of their circadian rhythm and examine effects on performance. We will then analyze effects of manipulating the LC system on the performance deficits produced by sleep deprivation.

Impact of findings

The development of a target detection task for the rat now allows us to test the effects of stressors on a type of performance important in space missions. This model will also allow analysis of the effects of manipulations of the brain NE system of the LC in these stress effects to facilitate development of countermeasures that should facilitate performance in the face of stress. We found that acute stress increases FA errors in this task, and that decreasing neurotransmission in the LC system with clonidine may offset this effect. Accordingly, we also found that increased NE neurotransmission (with idazoxan) in non-stressed animals worsens performance on this task by producing the same type of errors (FAs). These results indicate that the LC-NE system may be a valid target for development of countermeasures to the effects of stress on performance. Finally, we have developed a device to sleep-deprive rats and measure effects on performance in this task. This will allow analysis of this important stressor on performance, and the ability of manipulations of the LC system to offset such stress effects on performance.

Proposed research plan for next year

Studies will continue in the areas described above to confirm findings to date. In addition, new studies will be undertaken to examine the effects of chronic noise stress, as well as sleep deprivation stress, on performance. The ability of manipulations of the LC-NE system to offset these stress effects on performance will be determined.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Joseph V. Brady, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Psychosocial Performance Factors in Space Dwelling Groups

Project Executive Summary

The original aims of the project were the development and laboratory testing of a simulation approach for providing an automated means for research analysis of performances in space-dwelling groups as well as for monitoring electronically the effects of varying experimental conditions that influence psychosocial interactions. The significance and relevance of the research resides in the potential for conceptual and methodological advances that not only promote psychosocial and ecological stability in small isolated space-dwelling groups but may as well ultimately benefit larger societal units, including those that remain Earth-bound, by enhancing educational and training technologies that facilitate communication of an expanded generalizeable knowledge base.

With the shift in focus to long-duration manned spaceflight missions, planning strategies and tactics, as they relate to the behavioral biology science and technology support base, require reorientation and an essential change in emphasis. Whereas screening, selection, and training have been the hallmarks of demonstrably successful short-term manned space flight initiatives to date, the integration of behavioral and environmental programming systems "in flight" over extended mission durations must clearly be a priority concern in future spaceflight operations. Virtually all human life support functions (e.g., sleep and wakefulness, nutrition and fluid balance, work and emergency performances, organizational functions, social and recreational activities, etc.) will require operational definition in terms of behavioral interactions between organism and environment under conditions involving time series changes over both short and long duration intervals. A science-based technology for systems management, monitoring, and program control of such behavior/environment interactions is as essential to insure the success of long-duration manned spaceflight missions as is the hardware and software technologies that make such initiatives possible.

Simulations are at best approximations and all essential features of the operational setting cannot be replicated. Superordinate objectives and ultimate aversive consequences are, of necessity, less compelling under laboratory communication circumstances, and many organizational and sociopolitical issues of critical concern in considering the needs of projected long-term space-dwelling groups must await more advanced phases of such an investigative endeavor. But the obvious benefits of obtaining valid and reliable answers to at least some of the operational communication questions of critical concern in planning and carrying out projected extended manned space missions (e.g., command structure and functions, group performance effectiveness, work and social interactions, etc.) far outweigh the proportional costs when the magnitude of the resource investment required for these initiatives is taken into account. And even if these extended duration human operations in space were to be postponed well into the future, the lead-time to get our ducks in a row on these behavioral biology requirements clearly emphasizes the need for long-term human studies under experimentally controlled laboratory conditions.

Progress previously reported on the research accomplishments during the initial award year ending on January 31, 2002 focused upon the development and pre-testing of the programming software for a three-person crew simulation of the planetary exploration mission involving an Orbiter/Lander/Rover model. The computer hardware was assembled to permit operational interaction between the communication and expeditionary software systems on the networked simulated crew stations. Construction of a specially designed laboratory was completed and provision was made for three acoustically controlled workstations plus a separate 'Mission Control' station for implementation and operational management of the research simulation. Recruitment and training of essential technical and research support personnel as well as troubleshooting of both the software and hardware systems begun during the initial award year was advanced during the current award period to permit experimental protocol design, recruitment of volunteer simulation astronaut crews and preliminary data collection.

Three groups of three crewmembers each (Orbiter/Lander/Rover model) participated in a total of 19 simulated 'Planetary Missions' (52 'Flights') with the objective of identifying, collecting, and analyzing geologic specimens with a range of graded values. Each 3-to-4 hour 'Mission' consisted of multiple 60-90 minute 'Flights'. Baseline conditions provided for the availability of all communication modalities (i.e. text messaging, drawing on shared 'white board', active video images, and audio vocal exchanges) between and among the crewmember components and 'mission control', and for the quantification of the collected geologic specimen grade values during control flights. The extent to which the interacting crew members could maximize each of the simulated spaceflight crew's psychosocial performance effectiveness was dependent upon utilization of the distributed communication modalities to exchange information about the location, identifying characteristics, storage, and analysis of the grade-valued geologic specimens. The effect of communication constraints (i.e. modality-specific 'system failures') on psychosocial performance effectiveness was determined by disabling individual and combined modes of communication (e.g. audio, text, white board) during selected Flights in the course of recurrent experimental Missions.

The results obtained with all three experimental groups confirmed that cooperative and productive psychosocial interactions could be maintained between individually isolated and dispersed members of simulated spaceflight crews communicating and problem-solving effectively over extended time intervals without benefit of one another's physical presence. Experiments involving communication modality constraints showed clearly that, with the scenarios tested, there was a high degree of interchangeability between the available modes of communication. Psychosocial performance effectiveness was well maintained even under conditions involving multiple modality constraints (e.g. disabling both text and white board). With all three groups, the changes of greatest magnitude were observed in text messaging increases under conditions involving reduced utilization of audio communication. Moreover, there were indications of performance enhancement effects with some crews (i.e. increases in total geologic specimen grade values) during Flights reflecting extensive text messaging, compared to Flights under conditions involving either constrained access or reduced utilization of alternative communication modalities (e.g. audio, white board, etc.).

The completion of the above studies set the stage for investigating the effects of experimental manipulation of both positive and negative incentive conditions as well as experimentally induced time pressure stress. Both positive and negative methods for maintaining on-task time were evaluated via additions to and subtractions from a group incentive "bank account" as related to the consequences of success or failure in accomplishing simulated space-dwelling group task completion requirements. Time pressure was used as a stressful condition by adding

unscheduled “radiation storm drills” that interrupted task activities and created timing delays in completion schedules within and between simulated scenario phases. On the one hand, additional time pressure, as induced via radiation storm drills, results in dramatic decreases in psychosocial performance effectiveness. On the other hand, the effects of added positive incentives as a counter-measure for time-pressure stress reverses such declines in performance effectiveness, thus suggesting itself as an effective countermeasure to stress-related reductions in psychosocial performance effectiveness.

The generalizability of these findings and the development of effective countermeasures to observed performance decrements remain to be evaluated in future experimental studies based upon variations in scenario complexity as well as additional time stress and incentive conditions as they affect distributed interactive communication and psychosocial performance effectiveness.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	James A. Carter, Ph.D.
ORGANIZATION:	Harvard – Beth Israel Deaconess Medical Center
PROJECT TITLE:	Designing a Smart Medical System for Psychosocial Support

Project Executive Summary

Project Aims

This project involves the prototyping and evaluation of a computer-based system to assist astronauts in preventing, assessing and managing psychological and social problems that can arise during long-duration space missions. In the prototype, we are addressing the problem areas of depression and conflict, drawing on the experience and expertise of veteran long-duration flyers as well as national experts in conflict resolution and depression treatment.

Specific aims of this project include:

1. Develop the architecture of a prototype Smart Medical System for Psychosocial Support
2. Develop a prototype computer-based system for self-assessment of depression
3. Develop a prototype computer-based system for the self-treatment of depression
4. Develop a prototype training module on conflict resolution
5. Evaluate the system for usability, acceptability, and perceived value with astronauts and experts in conflict and depression

a. Project Phases

The project involves three phases:

1. Conducting consultation interviews with former long-duration flyers from the International Space Station, Mir, and Skylab, as well as experts in our research team in conflict resolution and depression treatment.
2. Producing a proof-of-concept prototype of an interactive Smart Medical System for Psychosocial Support, including the basic interface and the frameworks of programs on depression and conflict management.
3. Evaluating the system's usability, acceptability and perceived value to astronauts.

b. Consultation interviews with veteran long-duration flyers.

Interviews with veteran long-duration flyers were an essential early step in developing the prototype because they are the best subject matter experts on the psychosocial experience of long-duration space flight. Interviews with these individuals provided value in the following ways:

1. Understanding the psychosocial environment
2. Identifying best practices for managing psychosocial problems on long-duration missions
3. Choosing and suggesting simulations to develop
4. Suggesting courses of action to take in simulations
5. Differences between Earth and space
6. Introducing the study to long-duration flyers to solicit their support and involvement

7. Identifying interviewees to be taped for use in the system

Participants were presented with five fictitious scenarios dealing with depression and conflict in space and asked for their opinions on:

- The best approaches (best practices) to managing each situation as a commander
- The best approaches (best practices) to managing each situation as a non-commander
- Undesirable actions to take in each situation
- Other options that might be available to deal with each problem
- The realism of each scenario
- Improvements that could be made to each scenario for training

Key Findings

Phase I has been completed, which involved securing IRB/CPHS approval from NASA-Johnson Space Center to interview space and ground crew personnel (a 6-month process), and conducting 11 interviews with veteran long-duration flyers: from the ISS (n=2), Mir (n=5), and Skylab (n=4) programs.

The interviewees provided a wide range of opinions, occasionally contradictory, regarding the best ways of managing the problems in the scenarios and pitfalls or missteps that might be made. Although all scenarios were judged realistic by the majority of participants, there were elements of some scenarios that did not ring true or could be improved. We gained insights into elements that should be added to the scenarios to enhance realism and will select one of these scenarios to develop into a full simulation. Finally, while one of our goals is to get concrete information on responses to scenarios, we are also obtaining a considerable amount of information about the “art” of dealing with problems on long-duration space flights that is hard to capture in a table, chart or graph but can be incorporated into the countermeasure through videotaped interviews with veteran flyers and simulations involving actors.

Across all scenarios, a general pattern of best practices emerged for *commanders* helping fellow crewmembers manage conflict and depression:

1. Discuss the problem with the affected crewmember
2. Try to help resolve the problem, with actions dependent on the nature of the problem
3. Consult with ground crew, with the affected crewmember’s consent. Contact the ground without crewmember’s consent if problem is serious or persistent
4. Involve the crewmember in problem-solving
5. Take an authoritative stance when needed, stressing that the mission is the crewmember’s top priority or giving ultimatums
6. Assess problems from multiple angles to find causes
7. Serve as a go-between for crewmember and ground

The following best practices pattern emerged for *non-commanders* helping fellow crewmembers to manage conflict and depression:

1. Discuss the problem with the affected crewmember
2. Refer the problem to the ground, with crewmember’s consent, if it is a serious or persistent problem
3. Identify the causes of the problem
4. Take action to fix the problem, such as sharing workload, or increasing communications with family, depending on the nature of the problem

Inappropriate actions, or pitfalls, that were most frequently indicated were:

1. Talking to the ground about another crewmember's problem without his or her consent—making the problem known outside of the space crew
2. Doing nothing about a problem
3. Blaming the crewmember for having the problem, considering him or her to be lazy, et. cetera
4. Not noticing problems or not appreciating their significance
5. Overreacting to problems

Impact of Findings

Findings from consultation interviews with long-duration flyers will directly inform the development of the Smart Medical System for Psychosocial Support by:

- Helping to identify appropriate and inappropriate responses to include in simulations to be developed for the System
- Helping to identify ways in which conflict and depression must be managed differently on long-duration space flights compared to on Earth
- Enabling us to integrate best practices from the perspectives of long-duration flyers and experts in depression and conflict management, ensuring that the program is based on empirically validated supported methods as well as being acceptable to astronauts
- Introducing veteran flyers to the project, to enlist their future support
- Identifying potential interviewees who may be videotaped on camera discussing best (and worst) practices to managing conflict and depression in space

Research Plan for Year 2

We will attempt to complete this ambitious study on schedule, in Year 2. Year 2 will involve the production of the prototype in with support from The Troupe Modern Media in Windham, New Hampshire, and evaluation of the prototype at NASA-Johnson Space Center.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Optical Computer Recognition of Behavioral Stress

Project Executive Summary

The goal of this project is to develop and test an optically based computer recognition algorithm of the face to reliably detect the presence of stress during performance demands. Manned space flights of increasingly longer durations are being planned. There is evidence from U.S. and Russian space missions that astronauts and cosmonauts have experienced operational stressors that adversely affected subjective well-being, physiology, and performance capability. In order to provide countermeasures for stressor-induced impairments in astronauts, objective, unobtrusive measures of the presence of stress reactions are needed. This project seeks to achieve such a measure through collaboration between laboratories with expertise in the evaluation of behavioral and physiological responses under stressful and non-stressful conditions (Prof. D. Dinges, Department of Psychiatry, Unit for Experimental Psychiatry, The University of Pennsylvania), and in optical computer recognition of human subjects' facial expressions and gestures (Prof. D. Metaxas, Computer Sciences, Rutgers University).

Astronauts aboard extended-duration space missions will endure the harsh space environment and the effects of various stressors (e.g., microgravity, perceived risks, work requirements, habitability constraints, radiation, restricted communication with Earth) to a much greater degree than have been experienced previously. Maintaining individual neurobehavioral functioning of astronauts will be vital to assuring mission success. However, in order to provide countermeasures for stressor-induced, physical and functional impairments in astronauts, objective measures of the presence of heightened stress reactions are needed. The earlier that stress reactions (regardless of their operational, psychosocial, or neurobiological source) can be detected, the greater the probability that an appropriate countermeasure strategy can be implemented (e.g., rest, pharmacology, behavior). In the absence of objective detection of developing stress reactions, it is unlikely that countermeasures for stress impairment of astronauts can be managed. Many techniques for monitoring stress reactivity in space flight are impractical (e.g., cortisol measurement), unreliable (e.g., self-report), or obtrusive. However, unobtrusive, continuous video monitoring of the human face during neurobehavioral tasks, offers a potential solution to these problems. Consequently, this project will provide the first scientific test of the use of optically based computer recognition of the face to unobtrusively and reliably detect the presence of stress during laboratory performance demands.

The proposed computer-based optical recognition system will build on the research of Prof. Metaxas by utilizing automatic optical tracking of human subjects' anatomical and motoric changes in facial expressions during non-verbal performance tests. Video input to the system will be provided from experiments performed in the laboratory of Prof. Dinges, in which healthy adults (males and females of different ethnic backgrounds) will be exposed to behavioral stressors to increase the likelihood of developing a sensitive algorithm.

The aim of the protocol is to experimentally establish whether an optical computer recognition algorithm based on facial expression can be developed that can objectively, independently and reliably discriminate when subjects are undergoing behavioral stressors, and whether a high

degree of accurate categorization can be achieved for both male and female subjects; for both younger (22-32 years) and older (33-45 years) subjects; and for subjects of different ethnic backgrounds. Further, in exploratory and heuristic analyses, we will evaluate the effects of behavioral stressors on physiological responses of cortisol secretion and heart rate, on psychological responses of self-report ratings of stress and mood, and on neurobehavioral performance responses; and explore the extent to which the magnitude of the stress response as assessed by these measures relates to the accuracy of the optically based computer recognition algorithm of the face.

A single-blind, repeated-measures controlled trial will be used to achieve these aims and to provide the data required to test the hypothesis that an objective, unobtrusive, optically based computer recognition algorithm of the face can be developed to reliably detect the presence of high stress (and of low stress) during performance. A total of 60 healthy adults will be studied in the Unit for Experimental Psychiatry laboratory (Dr. Dinges) during a single testing day, consisting of three sessions: I—screening session; II—training session for development of the optical computer recognition algorithm; and III—prospective test session of the predictive utility of the optical recognition algorithm to discriminate high versus low stressed states associated with behavioral stressors. Stress reactions will be tracked during both control (low stress) and high stress conditions in sessions II and III, by measurement of salivary cortisol, heart rate, subjective mood/stress responses, and neurobehavioral performance. Videos of subjects' faces in the low and high stress conditions of session II will be used by the Vision Analysis and Simulation Technologies laboratory to develop a predictive optical algorithm that will be tested blind to stressor level (i.e., high vs. low) in the behavioral stressor conditions of session III.

The experiment is designed to test the hypothesis that an optical computer recognition algorithm can be used to discriminate when subjects are undergoing behavioral stressors, as defined by established stress-related changes in cortisol secretion, heart rate, subjective reports, and performance.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Stephen M. Kosslyn, Ph.D.
ORGANIZATION:	Harvard College
PROJECT TITLE:	Quick Assessment of Basic Cognitive Function: Blood Pressure Cuffs for the Mind

Project Executive Summary

This project is designed to develop a "tool box" for assessing the efficacy of specific aspects of cognitive processing. A number of cognitive tasks will be presented by a hand-held Palm OS computer very quickly, allowing a fast and accurate "read out" of a person's processing abilities at that moment. Our aim is to design short, easy-to-administer tests that will assess very specific types of processing. Automatic data analysis has been programmed into the Palm application, so that scores can be available immediately (or can simply be stored, for later analysis; all scores will have time and date stamps).

The first year of the project focused on developing the Palm OS application, dubbed MiniCog, and in the scripting of a number of short cognitive tasks to be administered by this program. We commissioned Bay Area Software to write the code for both the handheld application and a desktop interface. Bay Area Software is run by Sam Kho, who was the chief programmer for the M100 series of Palm Pilots, and his associate, Jolly Chen, also a programmer for Palm, Inc.

Tasks are scripted using an off-the-shelf HTML editor, and then converted to a Palm OS compatible format using an interface provided by Bay Area Software. MiniCog is designed to read task scripts, present stimuli for specific amounts of time, and record responses and response times, along with a time-and-date stamp and basic user info (ID, date of birth, sex, and handedness). When a task is complete, users are prompted to enter a password to see a display of their current score (as a function of response time, error rate, or variance, compared to norms). All data are stored for later upload to a desktop computer where more detailed analyses can be performed. The tasks differ only in the instructions (which are presented in initial screens), stimuli, and the number of response keys used.

MiniCog is flexible, allowing one to program new tasks quickly and easily as the need arises (e.g., to test a specific aspect of functioning that is needed for a particular real-world application). However, the current version has the following limitations: 1) Only visual stimuli can be presented; 2) the stimuli are presented either in a random order or in a fixed order (adaptive testing is not possible); 3) the responses are limited to key presses (up to 6 keys can be used).

Based on the literature in cognitive psychology and cognitive neuroscience, we have selected an initial set of tasks that taps key information processing. The tasks themselves are modeled after corresponding tasks in the literature. The specific tasks we have implemented assess attention (vigilance, divided attention, and filtering), motor control, verbal and spatial working memory, and verbal and spatial reasoning.

Two major developments emerged from this year's work. First, we now have a battery of tasks for which we are about to obtain norms with healthy subjects in standard laboratory conditions, and will also begin to explore which tasks are affected under experimental conditions (for instance, sleep-deprivation). Second, we now have an application that could be used by other

researchers to develop their own psychological tasks. Both the MiniCog application and our set of tasks have several crucial advantages over many typical psychological experiment scripting programs and standard tests: the tasks themselves are very short, and the method of administration is extremely compact and portable (compared to test packages containing booklets, answer sheets, timers, writing utensils, etc.) and inexpensive (compared to standard desktop or laptop computers).

The second year of the project will be devoted to acquiring task norms under laboratory conditions, as well as to refining the MiniCog application and developing additional tasks as needed.

We anticipate that the final version of MiniCog and accompanying task battery will be useful for ground-based research and applications as well as for spaceflight. (Temporary cognitive deficits occur under many situations, for instance during sleep-deprivation and high-stress situations. Surgeons, pilots, and truck-drivers may benefit from a quick cognitive check as well as astronauts.)

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Philip Lieberman, Ph.D.
ORGANIZATION:	Brown University
PROJECT TITLE:	Speech Monitoring Cognitive and Personality Alterations

Project Executive Summary

Original Aims

Our long-term goal is a system that will detect cognitive deficits, changes in personality and emotional disturbances occurring during prolonged space flight by means of acoustic measures of a person's speech. The system that we propose would monitor the flow of normal conversation, deriving relevant acoustic parameters using automated computer-implemented procedures that we will develop and verify in this study. We have studied the speech and behavior of individuals in a "space-analog," as well as patients suffering neurodegenerative diseases. These populations, to different degrees, exhibit degraded neural processes regulating these behaviors, that may also be compromised in space.

Our previous studies demonstrate that certain cognitive deficits can be monitored by means of objective acoustic measures of speech (Lieberman et. al., 1992, 1994, 1995; Pickett, 1998; Pickett et al., 1998; Lieberman, 2000). Other studies suggest that speech measures may provide indices of personality alterations. Advances in neuroscience show these dependencies reflect the neural architecture of the human brain. Dopaminergic, subcortical basal ganglia structures regulating speech motor control also support cortico-striatal neural circuits regulating cognition. Altered activity in these circuits can result in apathy, irritability, disinhibition, mood changes and obsessive-compulsive behavior (Alexander et al., 1986; Laplane et. al., 1989; Cummings, 1993; Marsden and Obeso, 1994). Given the possibility of cosmic ray damage to cerebral dopamine pathways during long space missions, noninvasive, unobtrusive monitoring of space crews by means of speech analysis may be prudent and useful. We proposed a two track approach to achieve timely development of an operational system.

We are obtaining data using the "space-analog" studied in our previous NASA sponsored project, climbers ascending Mount Everest. The expeditions members selected have been a reasonable match to space-flight crews: extremely fit, highly motivated, and intelligent. It would be difficult to otherwise ethically reproduce the hypoxic insult, high levels of stress resulting from a life-threatening environment, and the resulting group interactions that occur on Mount Everest. Prolonged exposure to extreme altitudes during the Everest climb produces speech production and cognitive deficits (Lieberman et. al., 1994, 1995) similar in nature to those occurring in Parkinson's Disease, which degrades dopaminergic cortico-striatal circuits (globus pallidus, the principal basal ganglia output structure, is sensitive to oxygen deprivation). Whereas our 1993 study focused on one acoustic parameter derived from isolated test words, we have developed additional measures and verify techniques that will ultimately allow the implementation of a system that can analyze conversational speech. We also have assessed changes in mood and personality and speech measures that may track these changes. In addition, we are studying a population having more extreme compromised dopaminergic circuit function similar in nature to that which may occur in space, Parkinson's Disease patients.

Key Findings

We have replicated the findings of our 1993 Everest space-analog study which was limited to five subjects with an additional 40 subjects. In that study we showed that computer implemented acoustic analysis of a person's speech could show whether the person was suffering a cognitive deficit that would degrade decision-making ability. The acoustic measure did not reflect the content of a person's speech; it instead indicated the presence of deficits in speech motor control. During the scope of the present project we intend to expand the battery of cognitive tests in synergy with Professor Steven Kosslyn's NSBRI research group at Harvard University – the "Mini-Cog" test battery implemented on a Palm Pilot PDA. The Mini-Cog test battery was used in our April-June 2003 Everest study and preliminary analysis of the data has yielded valuable insights.

We have derived two more robust speech measures that track cognitive deficits – the "mean VOT separation" and the mean vowel duration. The vowel duration measure could readily be implemented in an on-line monitoring system. These measures have "hit" values exceeding 85 percent in signaling the presence of cognitive losses in hypoxic (oxygen deprived) subjects. We have also shown that we can detect extreme life-threatening cognitive dysfunction and personality shifts induced by hypoxia. The Everest climber in question developed profound speech motor sequencing deficits that resembled those occurring in individuals who had suffered major destruction of basal ganglia. He also showed an inability to change the direction of his thought processes similar to those occurring with major degradation of basal ganglia function. He was advised of his condition, but rejected all advice (a personality shift) and refused to change his climbing plan. We had no control over his actions (our Everest subjects are volunteer free-agents) and he proceeded to climb upwards through a major storm and perished two days later.

Two recent independent studies confirm that hypoxia damages the basal ganglia structures that would also be degraded by exposure to cosmic rays during deep space flight – the basic motivation for both our Everest space-analog and Parkinson's subject populations. The MRI brain-imaging study of Jeong et al. (2002) of a climber who suffered acute mountain sickness shows damage limited to globus pallidus – the principal output basal ganglia structure that would be degraded by prolonged exposure to cosmic rays in space flight. The MRI and behavioral study of Kuoppamaki et al. (2002) shows that initial damage to globus pallidus from hypoxia becomes progressively worse with the passage of time, leading to profound motor control deficits.

In our concurrent study of Parkinson's Disease subjects we have used an advanced eye-tracking technique that has verified the supposition that cognitive processing slows down during sentence processing in the presence of basal ganglia damage.

Our findings confirm the original hypotheses and suggest new applications – monitoring for motor and cognitive deficits deriving from sleep deprivation, and the relevance of these techniques for the diagnosis and treatment of neurodegenerative diseases such as Parkinson's.

One of the benefits of our research are its potential and present applications to the general public. Our procedures have already been applied to evaluate new treatments for Parkinson's Disease. We intend to pilot a study of verbal apraxia – a condition that affects the linguistic and cognitive development of young children. A survey of present findings suggest that the underlying problem is basal ganglia dysfunction similar in nature, though less profound to that seen in Parkinson's disease, perhaps at the level of hypoxia. In our current proposal for the next phase of

this project we hope to apply speech analysis techniques to the study of task-induced stress in collaboration with David Dinges NSBRI group at the University of Pennsylvania.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Judith M. Orasanu, Ph.D.
ORGANIZATION:	NASA Ames Research Center
PROJECT TITLE:	Distributed Team Decision Making in Exploration Missions

Project Executive Summary

Background and Project Goals

Successful long-duration space missions will depend on the ability of team members (both space crews and ground controllers) to respond to unanticipated problems and to collaborate effectively under highly stressful conditions. In addition to environmental threats (radiation, microgravity) and task-related stressors (time pressure, danger, workload, and fatigue), crews are subjected to psychosocial stressors such as confinement with the same small group of people, lack of privacy and personal space, isolation from family, and restricted or delayed communication with Earth. During prolonged space missions, these psychosocial stressors could well threaten the psychological well-being of the individual astronaut and limit crew effectiveness. While crewmembers are highly selected and technically skilled, "the history of space explorations has seen many instances of poor interpersonal relations and faulty decision making" (Committee on Space Biology and Medicine, NRC, 1998). As a result, NASA has concluded that interpersonal difficulties could well threaten the success of long-duration space missions (NASA Critical Path Analysis, [<http://criticalpath.jsc.nasa.gov>], NSBRI-99-02). Interpersonal relationships are of particular concern as crewmembers become more diverse in terms of culture, gender and professional backgrounds. Tensions are likely to result from miscommunications and misunderstandings based on differing cultural norms and expectations.

The goal of our project is to understand how team problem solving and decision making are affected by task-related and psychosocial stressors similar to those that may be encountered in space. Results of the study will yield a basis for (a) designing or revising procedures, training practices, and technologies to support effective team performance, and (b) developing technologies for monitoring and predicting breakdown of team interactions so that countermeasures may be introduced before team dynamics deteriorate to the point of threatening mission success. Five specific questions will be addressed in our studies: (1) What is the impact of task-related and psychosocial stressors on decision making and interactional behaviors in team problem solving situations? (2) What task and team strategies are most effective in performing collaborative work? (3) How do gender and cultural background influence decision making strategies and team interactions? (4) Are there physiological and biomedical indicators that predict individual stress and deteriorating team interactions? (5) Are these measures robust across gender and national group?

Approach

A set of experiments will be conducted to answer these questions. Teams of four participants will work together on a computer-based dynamic decision making task. While participants are working on the task, we will monitor their physiological responses and facial expressions for signs of mental and emotional stress. Participants' physiological arousal levels and facial affect will subsequently be correlated with individual and team performance in the decision making task. The distributed decision making task used in our studies involves a simulated search and rescue mission in Antarctica. The simulation presents graphical displays of evolving problem

scenarios and supports communication among team members via e-mail messages. The simulated mission will enable us to examine collaborative behaviors among team members in two phases of the task: (a) during *planning* of how to go about locating the lost party, and (b) during *executing* the search task under a variety of task constraints such as time limitations, unexpected obstacles, and unreliable information. The software will also permit manipulation of a variety of potential stressors in two categories: *Task Difficulty* and *Team Conflict*.

Accomplishments and Key Findings - Year One

Scenario developed for the simulated search and rescue mission. Aptima Inc. developed a prototype scenario for the search and rescue task. This prototype was tested in our lab for usability and software problems. A number of changes concerning the display and the search and rescue task have been requested of Aptima and are currently being incorporated. The first scenario in its four versions is expected to be completed and delivered to NASA Ames by the end of August. Two versions of the scenario will be designed to induce cooperation between team members and will vary in task difficulty (moderate and high). The other two versions will include variables to induce team conflict (again, one of moderate and the other of high task difficulty). Two additional scenarios (four versions of each) will be developed and delivered by September 30, 2002.

Laboratory Established. All the computer and video equipment necessary for our research has been purchased and set up. Four individual work stations have been connected through a local area network to enable team members' cooperation during the simulated search and rescue task. Physiological monitoring devices (Biologs developed by UFI) available at NASA Ames Research Center have been adapted for use in our experiments. Equipment and software required to time-stamp and coordinate the recording of dependent measures from various sources (i.e., behavioral, physiological and facial affect measurements) have been acquired and are currently being tested.

Physiological Measures. The biomedical literature was reviewed to identify physiological measurements that might be useful for analyzing mental and emotional stress. Seven candidate measurements were selected and included in a preliminary study to determine which of them were most sensitive to low levels of emotional and mental stress and thus best suited for early detection of stress. We also sought to reduce the number of measurements from seven to four in order to minimize subject discomfort. A final issue was whether physiological measurements taken at alternate locations on the body would provide meaningful data while allowing subjects maximum movement and comfort.

Physiological data were collected while participants, four healthy adults aged 21-39, engaged in a commercially available active video game intended to activate the sympathetic nervous system. Each trial began with five minutes of relaxation time, followed by 60 minutes of playing the video game. Upon completion of the game, the subjects were again given five minutes of relaxation time. In order to maximize movement and comfort and to receive stronger signals, the PPG electrodes were moved from fingers to earlobe, EMG electrodes were moved from the lower arm to the neck muscles, and SCL electrodes were moved from the hand to the foot.

Analyses of the sensitivity of the physiological measures while crews engaged in a stressful task compared to rest periods indicated that ECG (R-R interval, Heart Rate), EMG (frequency power spectra and the amplitude within the band width of 10-25 Hz), respiration (frequency and amplitude), temperature and SCL (trend measurements) proved to be the most useful measurements for assessing mental and emotional stress at a low level.

Implications

- Findings on the effects of stressors on team performance in a dynamic challenging task will extend the research base on stress and behavior. Types of performance that may be affected include problem solving task performance, communication, and cooperative behaviors.
- The combination of selected physiological measures and facial affect measurements (described below) may yield a powerful instrument for assessment of low level mental and emotional stress. If successful, this tool could lead to early introduction of countermeasures, and thus prevent development of high levels of stress and deterioration of team performance.
- Positive stress-coping behaviors in a distributed problem solving task will be extracted from our findings. These will serve as a basis for developing interactional and strategic behaviors for managing challenging team tasks. These will include communication, team support, cooperative and self-monitoring behaviors that can serve as the basis for countermeasure training.
- We anticipate testing laboratory-based task and stress-management techniques in non-laboratory conditions, such as simulations of long-duration missions involving multi-cultural crews (e.g., NASDA or ESA studies). Our goal is to adapt the tools so that they can be used by crews as self-monitoring and management systems.

Proposed Activities for Year Two

Facial Affect Coding. Our next research steps will include the synchronization of the selected physiological measurements with video recordings of facial expressions. We plan to focus on facial actions related to typical expressions of basic emotions. While each national culture has a different body language and behavior, facial expressions are relatively stable across cultures. For example, researchers at the Japanese Space Agency have successfully used the facial action coding system (FACS) developed by Paul Ekman (1971) to examine facial expression during long-term missions in the ISS. Based on experts' recommendations, we plan to use Ekman's emotional facial action coding system (EMFACS) as a basis for our analyses.

Data Collection. During the fall and early winter 2002, we will test the effectiveness of the task difficulty and the crew conflict manipulations, the reliability and validity of performance measures, and of our physiological and behavioral monitoring tools. These tests will be done with homogenous teams made up of US participants.

Full-scale data collection will begin once the tools, tasks, and manipulations have been tested and found to meet our requirements. Data collection will begin in the winter, 2002.

- The first study will address the effects of task manipulations on team performance using culturally homogenous teams. Teams will consist of all male subjects born in the US. Task difficulty and team conflict will be the independent variables in this study. Task difficulty will vary within team, while team conflict will vary between teams.
- Cultural diversity in teams will be examined in the second set of studies. Participants will be sought who are from countries that will participate in the ISS: Russians, Japanese, Canadians, Europeans and Americans. Only male subjects with technical training similar to mission specialists will be used. Teams will be configured as either culturally homogeneous or culturally diverse.
- Gender variation will be introduced in the third set of studies expected to begin at the end of year two.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	JoAnna Wood, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Individuals and Cultures in Social Isolation

Project Executive Summary

This study was designed to examine the roles of personality, culture, and group influences on behavior, performance, and health outcomes in winter-over Antarctic stations. The planned two years of data collection are nearly complete, and preliminary analyses of data have begun. Three preliminary findings are encouraging. (1) The Helmreich PCI appears to tap dimensions of personality not measured with the 16PF, another recognized research tool. (2) Female station leaders perceive significantly less social support from fellow expeditioners than do female subordinates, male subordinates, or male leaders. (3) There appear to be both individual and group characteristics that influence interpersonal tensions. These findings have significant implications for selection and training countermeasures.

**NSBRI RESEARCH PROGRAM
NEUROVESTIBULAR ADAPTATION**

Team Leader:	Oman, C. M.	MIT	
Associate			
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Cohen, M. M.	CO-I	NASA Ames		
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RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Jacob J. Bloomberg, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Understanding Full-Body Gaze Control During Locomotion

Project Executive Summary

Control of locomotion requires precise interaction between several sensorimotor subsystems. Exposure to the microgravity environment of spaceflight leads to post-flight adaptive alterations in these multiple subsystems leading to postural and gait disturbances. Countermeasures designed to mitigate these post-flight gait alterations will need to be assessed with a new generation of functional tests that evaluate the interaction of various elements central to locomotor control.

Traditionally, gaze stabilization has been studied almost exclusively as a problem of eyehead-trunk coordination. However, coordination between the eye-head and trunk may not be the only mechanism aiding gaze stabilization particularly during locomotion. Therefore the first goal of this study was to determine how the multiple, interdependent, full-body sensorimotor subsystems aiding gaze stabilization during locomotion are functionally coordinated. The second goal was to use this information to develop new tests of locomotion function to be used to evaluate the efficacy of countermeasures.

To address the first goal, two experiments were performed. In the first study (Study 1) we investigated how alteration in gaze tasking changes full-body locomotor control strategies. Subjects (n=9) performed two discreet gaze stabilization tasks while walking at 6.4 km/hr on a motorized treadmill: 1) focusing on a central point target; 2) reading numeral characters; both presented at 2m in front at eye level. The second study (Study 2) investigated the potential of adaptive remodeling of the full-body gaze control systems following exposure to visual-vestibular conflict. Subjects (n=14) walked (6.4 km/h) on the treadmill before and after they were exposed to 0.5X minifying lenses worn for 30 minutes during self-generated sinusoidal vertical head rotations performed while seated. In both studies we measured: temporal parameters of gait, full body sagittal plane segmental kinematics of the head, trunk, thigh, shank and foot, accelerations along the vertical axis at the head and the shank, and the vertical forces acting on the support surface.

Results from Study 1 showed that while reading numeral characters as compared to the central point target: 1) compensatory head pitch movements increased, 2) the peak acceleration measured at the head was significantly reduced, 3) the knee joint total movement was on greater during the period from the heel strike event to the peak knee flexion event in stance phase of the gait cycle. Results from Study 2 indicate that following exposure to visual-vestibular conflict changes in full-body strategies were observed consistent with the requirement to aid gaze stabilization during locomotion.

Taken together, results from Studies 1 and 2 provide evidence that the full body contributes to gaze stabilization during locomotion, and that different functional elements are responsive to changes in visual task constraints and are subject to adaptive alterations following exposure to

visual-vestibular conflict. These studies also successfully validate new integrated methodologies designed to assess locomotor function for countermeasure evaluation and validation.

To address the second goal of this study we developed a new test to measure dynamic visual acuity during treadmill walking. Astronauts returning from spaceflight experience reduced visual acuity during body motion of the kind experienced during walking due to alterations in gaze stability caused by neurovestibular adaptive changes. These changes in acuity have significant operational implications. The inability to see clearly during body motion can impair the ability to operate spacecraft, conduct EVAs and perform an emergency egress soon after landing following a long-duration spaceflight. Our newly developed dynamic visual acuity test allows us to measure changes both in static and dynamic visual acuity for both near (0.5 m) and far (4 m) visual target positions. This test was evaluated in both normal subjects and in patients with bilateral vestibular impairment. Results show a significant ability to reliably differentiate normal from clinical behavior.

We have used results obtained from this research to develop an in-flight measure of dynamic visual acuity. This test will measure static visual acuity while subjects stand on the ISS treadmill and dynamic visual acuity during treadmill walking. We are currently developing an integrated testing system using a computer driven microdisplay screen. This in-flight test will measure changes in static and dynamic visual acuity during the initial adaptation phase to spaceflight and during the full duration of the flight. This newly developed test will be performed on the ISS following delivery of the hardware via Shuttle flight ULF-2 in 2005.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	John L. Dornhoffer, M.D.
ORGANIZATION:	University of Arkansas for Medical Sciences
PROJECT TITLE:	Pharmacological Countermeasures for Space Motion Sickness

Project Executive Summary

Study Aims

Space motion sickness (SMS) is a problem during the first 72 hours of spaceflight and during transitions from different gravity environments. To date, there are no effective drug countermeasures that are able to combat SMS while allowing an individual to retain cognitive integrity. This creates a dilemma for astronauts as full cognition is particularly important during gravity transitions, such as take-off and landing. SMS is generally believed to be caused by a sensory conflict due to unweighting of the otolithic organs (i.e., vestibular cues indicate the head is stable while visual cues indicate the head is moving). We hypothesized that the vestibular dysfunction due to over stimulation of the semicircular canals by the rotary chair can serve as a paradigm for SMS, thus enabling us to effectively test drug countermeasures while using test batteries to determine the effect of these countermeasures on cognition.

Specific aim one determined the effects of four drug countermeasures (lorazepam, meclizine, promethazine, and scopolamine) in alleviating motion sickness induced by vestibular stimulation with a rotary chair. These countermeasures were selected based on our extensive clinical experience with pharmacologic interventions for vertigo. Specific aim two determined the effects of these countermeasures on cognitive performance and in counteracting the effects of rotation (our SMS paradigm) using an Operant Test Battery (OTB) to assess effects on short-term memory, learning, and time perception, and measures of the P50 potential to assess effects on arousal and distractibility (ability to filter out extraneous information, or sensory gating). Specific aim three, conducted at the Vestibular Function Laboratory in Antwerp, used 3-D oculography and unilateral otolith testing to determine the extent of correlation between vestibular dysfunction induced by the rotary chair and unloading of otolithic organs due to 0 G.

Key Findings

We have shown that only scopolamine effected a statistically significant mean change in duration of rotation compared to placebo ($p < 0.008$); scopolamine decreased the sensory gating deficit induced by rotation; scopolamine had one of the best cognitive profiles based on the OTB; and scopolamine exclusively affected the otolith organs (utricle system)

We have shown that over stimulation of the semicircular canals by rotation leads to decreased habituation to repetitive stimuli, as measured by the midlatency auditory evoked P50 potential, which may be at the root of a sensory gating deficit (an inability to appraise and filter out unwanted stimuli) present during SMS. In terms of alleviating the symptoms of rotation and the induced sensory gating deficit, our data in 72 subjects indicated scopolamine to be the countermeasure of choice. Scopolamine was the only countermeasure to effect a statistically significant mean change in duration of rotation compared to placebo ($p < 0.008$), with >40 percent increase in rotation time. Results with promethazine, the current pharmacologic treatment for SMS, were not statistically significant, and meclizine and lorazepam were no more effective than placebo.

Scopolamine by itself did not affect amplitude or habituation of P50 potential measures, suggesting that, at the dose used, scopolamine did not dysregulate RAS function to a significant level. However, scopolamine did lead to a lower decrease in habituation after rotation (~22 percent); in other words, scopolamine decreased the sensory gating deficit induced by rotation. Although this decrease was numerical and not statistically significant, it is indicative of a trend by scopolamine toward amelioration of the sensory gating deficit induced by rotation.

The Operant Test Battery (OTB) indicated the Delayed Matching-to-Sample (DMTS) task, or short-term memory and attention task, to be the most sensitive measure of cognitive performance. The DMTS indicated SMS by itself had no discernible effects on accuracy or response rate and that, at the doses employed, the rank order of drugs with the best cognitive profiles are meclizine > scopolamine > promethazine > lorazepam.

Work performed by our co-investigators at the Vestibular Function Laboratory in Antwerp using 3-D oculography and unilateral centrifugation for otolith testing confirmed the ability of our paradigm to accurately assess the effects of countermeasures on the vestibular apparatus, from which the sensation of SMS may originate. These studies showed that the different components of the vestibular system (the semicircular canals vs. the otolith organs) react differently to the countermeasures and that scopolamine exclusively affected the utricular response, indicating a possible mechanism of action for scopolamine via a direct effect on the utricular system.

The distribution of spin time frequencies among our study subject population (N=75) demonstrated that, in terms of the ability of our study subjects to tolerate induced SMS, the population had significant outliers. The majority of the study population exhibited a fairly low tolerance for induced SMS; however, there was a group of outliers who could spin significantly longer, appearing to have a predisposition to SMS tolerance. This novel finding, combined with results of off-axis rotation (see below), has been proposed for further examination as we study means of screening for SMS susceptibility and subsequent prophylaxis.

As an extension of our NSBRI study, we attempted to assess the otolith organs using a new clinical paradigm involving off-axis rotation and measurement of the subjective visual vertical (SVV), the ability of an individual in darkness to adjust a luminous line to true vertical while at rest and during rotation. Clinical assessment of otolith organs is important to space medicine research due to the involvement of the utricle and saccule in SMS. Our objective was to assess otolith function in subjects with no vestibular complaints and in subjects with unilateral labyrinthine hypofunction. Subjects with no vestibular anomalies were able to set the SVV very close to vertical. However, certain subjects showed a mild asymmetry (vestibular dominance) of the otolith organs during on-axis rotation that was further accentuated during off-axis rotation. In the vestibular patients, the SVV deviated significantly toward the side of the lesion. Some subjects with dominant otolith organs were able to spin in the chair two and a half to three times longer than those without ear dominance, indicating less susceptibility to motion sickness. By screening for ear dominance using the SVV/rotational paradigm, pre-flight medication or compensation behaviors could be instituted in those individuals with an indicated susceptibility to SMS.

Impact

This study addressed one of the main exploration-mission risk areas set forth by NASA in the Critical Path Roadmap (Impaired cognitive and/or physical performance due to motion sickness symptoms or treatments, especially during/after G-level changes [Risk Type III, Risk Rank 3])

and had a countermeasure readiness level of 6. We have addressed critical question 9.12: How effective are other drugs in providing fast relief in mission critical situations and does the drug have unacceptable side effects, particularly the short term effects on cognitive function? (Aims one and two). In addition, through the completion of this study, we have standardized measures of oculomotor function, postural stability, and cognitive performance (Aims one, two and three). These standards are crucial for establishing the effectiveness and quantifying the side effects of potential drug countermeasures.

During our two-year grant period, we have obtained salient findings in completion of the objectives of the original proposal. The results of our study have clearly indicated scopolamine to be the countermeasure of choice, leading to an NSBRI renewal proposal that will investigate optimization of the dose and delivery of this countermeasure. Pursuant to this, Dr. Lakshmi Putcha, senior pharmacologist at the Johnson Space Center and a member of the Smart Medical Systems Team, will serve as a co-investigator on the new project. Dr. Putcha has shown the bioavailability of oral scopolamine to be poor and quite variable compared to IV or intranasal administration and that oral scopolamine may not be effective as rescue therapy due to the effects of SMS on gut absorption and gastric motility. Thus, our renewal focuses on optimizing drug delivery parameters for scopolamine, testing potential combination therapies, and examining the feasibility of scopolamine as rescue therapy.

The results of our study will also advance earth medical research by determining the extent of correlation between rotary-induced motion sickness (i.e., vertigo) and SMS and developing a testable model that integrates our current knowledge of both conditions. This may ultimately help physicians treat patients with balance disorders related to inner ear dysfunction. Our findings at the Antwerp site strongly suggest that there is a distinct difference in reaction to medication between the semi-circular canals and the utricular system. This can have a great impact on the pharmaceutical treatment of dizziness and vertigo since different management might be necessary depending on the site (canal- or otolith-related) of the vestibular lesion.

Our comprehensive CNS assessment is currently being used to evaluate patients with tinnitus and vertigo, in an effort to determine if a pre-existing cognitive deficit underlies some of the symptom complex associated with these conditions (particularly fatigue, inability to concentrate, and depression). If such is the case, the optimized scopolamine regimen obtained in future studies would be applied as therapy. Demonstrating links between vestibular dysfunction and cognitive difficulties would be an important discovery by allowing clinicians to better educate patients about how vestibular pathology may affect their ability to concentrate and retain information. Our findings could also lead to future research into different treatment modalities. Current treatment for peripheral vestibular dysfunction includes the use of vestibular suppressants whereas our results may indicate that research is also needed in the area of treating patients. cognitive difficulties, possibly via CNS stimulants. Funding for this application is currently pending as part of an NIH COBRE grant.

Proposed Research Plan

Over this final year of our two-year project, we have completed our enrollment and testing, to give a total of 75 subjects for which data is available. We have submitted an NSBRI renewal (Optimization of Scopolamine as a Countermeasure for Space Motion Sickness.; PI: John Dornhoffer, MD [UAMS]; Co-Is: Edgar Garcia-Rill, PhD [UAMS], Merle Paule, PhD [NCTR], Paul Van de Heyning, MD, PhD [Antwerp], Floris Wuyts, PhD [Antwerp], Lakshmi Putcha, PhD [JSC]) that will examine alternate doses and delivery methods (oral vs. intranasal) for scopolamine, combination therapy, and utility of scopolamine as rescue therapy. We plan to add

the Psychomotor Vigilance Task (PVT) to our NSBRI protocol as a measure of basic attentional processes (behavioral alertness) in order to further validate our OTB findings and P50 results and to test for any learned behaviors. Studies conducted at the Antwerp study site will enable us to obtain better insight into the mechanism of action of scopolamine on the separate parts of the vestibular system as well as motion sickness and factors involved in SMS. We also plan to continue off-axis testing of the NSBRI study subjects in an effort to show a correlation between otolith symmetry and susceptibility to SMS, which could lead to a screening paradigm for astronauts and “tailored” SMS therapy.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Charles M. Oman, Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Visual Orientation and Spatial Memory: Mechanisms and Countermeasures

Project Executive Summary

When astronauts enter weightlessness, there is no sensation of falling, and normal simple head movements do not elicit disorientation and oscillopsia the way they often do in vestibular patients on Earth. However some astronauts experience persistent “inversion illusions”, and most crewmembers occasionally experience startling “visual reorientation illusions” when they leave their seats and float sideways or upside down, or simply even watch another person doing this. The illusion results from a sudden realignment of the cognitive reference frame used for spatial orientation, and a disorienting change in the subjective identity of interior surfaces (e.g. ceilings seem like floors). As a result, crewmembers make reaching errors, and can even become momentarily lost within the vehicle. These illusions – which crewmembers often call “the downs” - are known to trigger space motion sickness. 0-G disorientation is among the primary biomedical risks of spaceflight as defined by NASA’s Critical Path Roadmap. The goal of this multi-institutional, multi-investigator NSBRI neurovestibular research project is to better understand the process of visual orientation and spatial memory in 1-G and 0-G, and to develop countermeasures for these in-flight problems. Our specific aims are to study:

Human visual orientation. To better understand static and dynamic visual orientation illusions in 0-G by quantifying them in 1-G. To determine how visual frame, polarity, motion and gravireceptor cues influence the direction of the subjective vertical, the response of the oculomotor and motor control systems, stability of the visual world (oscillopsia), and how viewing one's own body, environmental brightness and color cues determine the subjective vertical. (L.Harris, I. Howard et al, York University)

Three-dimensional spatial memory and learning To understand why astronauts have difficulty making spatial judgments between modules with different visual verticals, by quantifying how humans use visual cues in 1-G to establish "spatial frameworks" with in and between adjacent visual environments. To develop a computerized technique for teaching generic 3-D spatial orientation and memory skills. To investigate and evaluate ISS allocentric coordinate marking systems, and to develop a "virtual porthole" display so trainees can learn to visualize the spatial relationships of ISS modules and potential escape routes in three dimensions. (C. Oman, et al, MIT/W. Shebilske, et al, Wright State)

Neural coding of spatial orientation in an animal model. To define how the preferred direction of limbic system head direction cell depends on visual, vestibular, gravireceptive, proprioceptive and motoric cues in a rat animal model during three-dimensional locomotion. To understand how the vestibular system contributes to these head direction cell responses. Ultimately, to develop a neurophysiological understanding of visual reorientation illusions and spatial cognition in astronauts. (J. Taube, et al, Dartmouth).

Our research team is an interdisciplinary group of psychologists, physiologists, and engineers, with background in visual, vestibular and motor psychology and physiology, human and animal

navigation and VR technology. We coordinate research through bimonthly teleconferences and inter-laboratory visits, and actively collaborate with other colleagues at NSBRI and NASA Johnson Space Center. Facilities include unique tumbling rooms at York University, animal research facilities at Dartmouth, and several types of immersive virtual reality facilities at MIT, York, and Wright State University.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Millard F. Reschke, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Modification of Eccentric Gaze-Holding

Project Executive Summary

Clear vision is a prerequisite for reliable performance of motor tasks. Space flight confronts the crewmember with a stimulus rearrangement that requires adaptation to function effectively with the new requirements of altered spatial orientation and motor coordination. Adaptation and motor learning driven by the effects of cerebellar disorders may share some of the same demands that face our astronauts. One measure of spatial localization shared by the astronauts and those suffering from cerebellar disorders that is easily quantified, and for which a neurobiological substrate has been identified, is the control of the angle of gaze (the "line of sight"). The disturbances of gaze control that have been documented to occur in astronauts, both in-flight and post-flight, can be directly related to changes in the extrinsic gravitational environment and intrinsic proprioceptive mechanisms thus, lending themselves to description by mathematical models. The basic models can be formulated using normal, non-astronaut test subjects and subsequently extended using centrifugation techniques to alter the gravitational and proprioceptive environment of these subjects. Further tests and extensions of the models can be made by studying abnormalities of gaze control in patients with cerebellar disease. Finally, tests of astronaut subjects during and after exposure to space flight, in association with the corresponding sensory-motor adaptations, will allow us to evaluate and extend our developed understanding of adaptation in the control of eccentric gaze-holding. The specific aims of this study are: (1) To investigate the mechanisms of gaze-holding in normal, non-astronaut subjects, with the head held in various orientations with respect to gravity and the head held in various orientations relative to both gravity and the trunk. This will involve characterizing the *time constant of centripetal gaze drift*, the rate in which the eyes naturally drift back toward the null position following an eccentric eye movement. (2) To investigate the mechanisms that adaptively compensate for gaze-holding failure, especially the "rebound nystagmus" phenomenon, which decreases the rate of centripetal drift of the eyes. We will study the time course of rebound nystagmus in normal, non-astronaut subjects. (3) To investigate the stimulus rearrangement and adaptation resulting from exposure to gravito-inertial environments *greater* than 1 G using prolonged exposure to centrifugation. (4) To study mechanisms that adaptively compensate for gaze-holding failure in patients with vestibular cerebellar disease who show impaired gaze-holding ability. We will compare gaze-holding defects and rebound nystagmus in patients with that obtained in our normal subjects. (5) To compare the gaze-holding abilities of astronaut subjects prior to, during, and immediately following space flight with specific predictions made as a consequence of the ground-based research. Tests similar to those performed upon normal, non-astronaut subjects will be conducted to quantify changes in the time constant of centripetal drift of the eyes in relation to changes in the gaze-holding induced as a result of the stimulus rearrangement of space flight. (6) To measure the stability of gaze, during all phases of flight, with the eye at the central position in astronauts to investigate the occurrence of saccadic intrusions known as "square wave jerks" (SWJ), and to relate SWJ mechanisms common to the failure of gaze-holding.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Mark J. Shelhamer, Sc.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Context-Specificity and Other Approaches to Neurovestibular Adaptation

Project Executive Summary

There are several operational issues involved with altered human performance during and immediately after space flight. These issues have implications for human safety and effectiveness. Our planned experiments are designed to give us the information needed to develop and assess appropriate countermeasures (pre-flight or in-flight activities) for the vestibular deconditioning that occurs during flight (and often persists upon return to a planetary environment). Whenever g-transitions occur, there is a very real possibility of disruptions in perceptual and sensorimotor processing and reflex calibrations. These can have serious consequences in a dynamic environment such as shuttle re-entry or Mars landing.

We propose context-specific adaptation (CSA) as a countermeasure to some of the deleterious neurovestibular effects of space flight. By CSA we mean the ability of an organism to 1) maintain two different adapted states for a response (such as two different saccade gains), 2) have each state associated with a specific context (such as g level), and 3) switch between the adapted states immediately upon a change in context (i.e., without de-adaptation and re-adaptation upon each transition). This phenomenon can be useful during phases of space flight that require transitions between different g environments (e.g., in and out of artificial gravity, from orbital flight to planetary landing). A related theme is the determination of effective adaptation procedures and effective context cues. The role of the cerebellum, and its possible disruption during flight, is another central issue, as is transfer of adaptation between motor systems.

Outline of sub-projects in this proposal

Our project consists of an integrated set of experiments that have as their overall goal the design of a spaceflight countermeasure based on forms of vestibular adaptation. Briefly, the experiments include three main investigations at Johns Hopkins: 1) studies on the effects of torsional misalignment, and the use of saccade adaptation and cyclovergence adaptation as countermeasures (Shelhamer/Zee, aims 1-3), 2) studies on the relationship between the LVOR and smooth pursuit and the role of the cerebellum on adaptation of these responses (Zee/Minor/Shelhamer, aims 4-6), and 3) a study on context cues in the human LVOR (Shelhamer, aim 9). Another set of experiments will be conducted at Washington University (St. Louis) to study how CSA might transfer between eye movements and limb movements (Angelaki/Snyder, aim 7), and experiments at the University of Mississippi Medical Center will investigate adaptation of the LVOR with transient accelerations (Zhou, aim 8).

General outline of the progress report

The research progress described in this report represents something of a major redirection of effort from the previous reporting period, as some projects wind down (Aims 7 and 8) and others focus on new findings (e.g., Aims 1-3 focusing on skew). We also take advantage of related ongoing work and its applicability to some of the neurovestibular problems of space flight (the LVOR stimulated by small rapid translations, vertical saccade asymmetries).

Specific Aims (as originally planned)

1. To determine if static torsional eye position (induced by a visual display or by parabolic flight) can be used as a context cue for the adaptation of saccade metrics. Previous work implies that torsional changes in flight may affect saccades and other spatially-oriented behaviors. We will attempt to demonstrate that saccades can be made veridical in two different torsional states.
2. To see if CSA can be more readily acquired by allowing consolidation of adaptation to take place before changing contexts. We will allow for consolidation of each adapted state to occur by inserting a rest interval between the two context states during the CSA procedure.
3. To develop cyclovergence adaptation as a countermeasure to torsional offsets during changes in gravity. A visual stimulus can be used to induce torsional misalignment (cyclovergence). We will design an effective cyclotorsion adaptation stimulus in lab experiments, and use it to maintain the usual (1g-based) torsional alignment during parabolic flight, and see if otherwise inappropriate responses (saccades) in flight are evoked correctly if torsion is “corrected” to its normal (1g) state.
4. To compare horizontal and vertical pursuit and LVOR deficits over a wide range of frequencies, in cerebellar patients and in monkeys with vestibulocerebellar lesions.
5. To study in normal humans, and in monkeys before and after vestibulocerebellar lesions, pursuit and LVOR adaptation and their transfer over a wide range of frequencies.
6. To study in normal humans, and in monkeys before and after vestibulocerebellar lesions, CSA of the LVOR and in particular the ability to use pursuit stimuli with different g cues as a stimulus for learning multiple LVOR gains as a function of the g state.
7. To determine if CSA learned in one behavior (eye movements) will transfer to a different behavior (arm movements) in rhesus monkeys. We will use static head tilt as a context cue to adapt either the horizontal AVOR or horizontal saccades. Then we will investigate whether this context-specific adaptation is also present in memory-guided saccades and reaching. Experiments will be performed in intact animals and in animals with cerebellar lesions.
8. To use the transient linear vestibulo-ocular reflex (LVOR) to study context-specific otolith-ocular adaptation in human subjects. Our goal is to find the most effective procedure for adaptation of the transient LVOR, in anticipation of its possible use as part of a space flight countermeasure. (a) Systematically characterize task-specific LVOR adaptation in human subjects. (b) Identify the most effective training protocols to induce context-specific adaptation in human subjects. (c) Test for the ability of visual cues to substitute for vestibular cues in context-specific LVOR adaptation in human subjects.
9. To study CSA in the naso-occipital LVOR as for the inter-aural LVOR, and to determine what context cues are effective in each case.

Key findings and their impact

1. Two negative findings are of interest. First, we found that there is not a noticeable vertical error in horizontal saccades in parabolic flight. We thought that there might be, based on separate findings of disconjugate torsion in altered g levels, and saccade errors with the eyes deviated torsionally. The fact that we did not find such an error in the altered g levels of parabolic flight suggests that we can rule out this aspect of saccade accuracy as a confounding

sensorimotor issue in space flight. The other negative finding is that asymmetries in vertical saccades (gain and latency) do not appear to be solely, or even predominantly, gravity-related. Again, this suggests that vertical saccades are likely not adversely affected during flight, at least as a direct consequence of altered g level.

2. A very significant and unexpected finding is the presence of vertical ocular misalignment (skew) during the altered g levels of parabolic flight. This was found in the course of the investigations on saccade error described above. It was first noticed when subjects reported that they saw a single small target light split into two (diplopia), and that they could not fuse the two images, especially in the 1.8 g phase of flight. This phenomenon may be another consequence (as disconjugate torsion likely is) of otolith asymmetry, and has clear implications for piloting and other tasks during g transitions.

3. Our various findings on the properties and adaptability of the linear VOR (LVOR) have increased our base of knowledge of this fundamental response. We hope to use this paradigm as a test of otolith and cerebellar function in the future. In particular we feel that it can be an important part of a standard pre-flight/post-flight vestibular test battery for flight crews.

Research plan for coming year

The overall plan for year three is substantially changed from that originally outlined. In particular, we intend to follow up intensively on our new findings on vertical skew (including its extent, adaptation, and visual consequences), and pursue the development of the LVOR as a test of otolith and cerebellar function.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Conrad C. Wall III, Ph.D.
ORGANIZATION:	Harvard – Massachusetts Eye and Ear Infirmary
PROJECT TITLE:	Advanced Techniques to Assess and Counter Gait Ataxia

Project Executive Summary

The overall goals of this project are to develop “countermeasure assessment criteria” to evaluate recovery from disturbances, and during turning, circular walking and ascending and descending stairs. We also consider countermeasures using a balance prosthesis and dynamic exercises designed to challenge and increase subjects’ balance. We will determine the sensitivity of the countermeasure assessment criteria in evaluating effects the prosthesis and the exercises on postural stability and locomotion. Using human subjects, the specific aims of this project are to:

1. Study body and head movements during precise perturbations of gait during continuous straight locomotion.
2. Study body, head and eye movements during continuous straight or circular locomotion on a circular treadmill.
3. Study body, head and eye movements during ascending and descending a staircase.
4. Study body, head and eye movements during standing, linear walking and treadmill walking with a balance prosthesis designed as a countermeasure for vestibular adaptation.
5. Study the effect of dynamic balance exercises for vestibulopathic subjects upon their ability to stand quietly and to recover from mild perturbations.

Key findings of the project

We have developed an experimental protocol that introduces a calibrated disturbance to the foot during the support phase of normal locomotion. This provides a means for the objective quantification of locomotor response dynamics that are known to be altered in astronauts upon return from exposure to microgravity but for which no current test exists. Returning astronauts whose orientation mechanism has been distorted and patients having balance disorders (vestibulopathies) that may well affect their orientation mechanism was expected to have different recovery trajectories than healthy normals. This is has now been demonstrated for vestibulopathic subjects. A simplified version of our research device is now being developed for use in evaluating the functional mobility of astronauts by scientists at the Johnson Space Center.

One of our working hypothesis was that profound impairments of posture, gaze and locomotion stability are caused by alterations in compensatory and orientation mechanisms that are generated in the central vestibular system from motion inputs. During exposure to altered gravity, the motion inputs from the otolith organs are “distorted” compared to the on-earth conditions. These distortions, in turn, cause both inappropriate body head and eye movements and an altered sense of orientation, which degrades stability during locomotion. We compared motions of the body during walking along a straight line with body motions while walking along a curved path. In the latter condition subjects accelerate in toward the direction of the curve, which introduces an inertial component which may or may not effect measures of their body orientation in space. Our results show that compensatory eye, head and body movements stabilize gaze during straight walking, while orienting mechanisms direct the eyes, head and body to tilts of the resultant of gravitational and centripetal acceleration in space during turning. This finding in normal subjects can now be compared to subjects with known impairments in their balance system or to returning

astronauts to determine whether or not such individuals can successfully align parts of their bodies in an appropriate way while turning.

We have developed the simplified precursor to a balance aid. It uses body mounted motion sensors to estimate the tilt of the subject. This estimated tilt is coded and fed back to the subject using an array of small, non-invasive tactile vibrators mounted on the skin. The application of vibrotactile display of body tilt demonstrates for the first time (to our knowledge) that tilt estimates derived from body-mounted motion-sensing instruments can actually be used to reduce sway in subjects who have documented deficits in their balance (vestibular) function. The single most important finding was that subjects who repeatedly fell under challenging balance conditions were able to stand with the use of this aid.

Impact of these findings on the hypotheses or requirements (technology), objectives and specific aims of the original proposal and the proposed research plan for the coming year

These findings indicate that the overall objectives of the project are being met. The research plan for the coming year will remain as originally planned.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Laurence R. Young, Sc.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation

Project Executive Summary

Traditional countermeasures against the adverse effects of prolonged weightlessness, such as exercise, resistive garments and lower-body negative pressure, appear to be insufficient in practice and are often too inconvenient for astronauts. Artificial Gravity (AG) represents a potential countermeasure that is unique. It promises salutary effects on bone, muscle, cardiovascular and vestibular function. Rather than alleviating the symptoms, it attempts to remove their cause. Spacecraft size dictates that any AG centrifuge tested in space in the foreseeable future be of limited radius (on the order of 1-5 m). In order to achieve sufficient centrifugal forces equivalent to 1-g, rotation rates will have to be rather high (between 10 and 30 rpm). Unfortunately, at these rates a number of side effects occur, including motion sickness, reflexive eye movements, and unpleasant sensory illusions. Fortunately, people seem to adapt to such sensory rearrangement changes. The matter is further complicated because our senses and motor system still need to function in zero-g and one-g as well as in AG. Thus, the astronaut must adapt to function effectively in at least two environments, centrifugation and zero-g. Dual or multiple context-specific adaptation is required. The goals of the present research project are to gain insights into how the motor and perceptual systems are able to adapt in this context-specific manner and to use these insights to develop practical AG countermeasure protocols. To meet this goal, we pursue eight specific aims forming a unified research program that consists of two categories. The first attempts to understand the basic mechanisms underlying context-specific adaptation. The second involves applied questions related to optimizing the conditions for adaptation. During the first year of funding we have made progress in both categories, we showed that AG is a promising candidate for a universal countermeasure.

During the second year, now nearing completion, we extended our experiments to the adaptability to more complex head and limb movements in the rotating environment. Among other experiments, we adapted subjects to making yaw head-turns on the MIT rotator. Once well adapted to the point of zero subjective illusion strength and zero motion sickness, the subjects were tested in the Brandeis rotating room. The physical stimulus was identical but the external environment was as different as can be. The adaptation transferred completely. This result indicates that adaptation is associated with the vestibular stimulus and not with circumstantial extraneous cues. This finding has implications for countermeasure development. We might be able to pre-adapt astronauts in one environment and assume that the adaptation transfers to a vastly different setup, such as from a ground-based centrifuge to one in orbit. We also tested the transfer from yaw head-turns in the right quadrant to yaw turns in the left quadrant. After an initial startle effect (subjective intensity ratings went from zero back to about six, the first head turn ever being a 10) upon switching the quadrant of the yaw turn, all subjects very quickly adapted to the new to the fullest. Subsequent quadrant switches did no longer upset the subject. We are currently investigating generalization from yaw adaptation to pitch and roll head movements.

At Mt. Sinai, progress was made showing that the anti-motion sickness drug promethazine did not interfere with adaptation to vertiginous stimulation. The dominant vestibular time constant reduced *pari passu* with the reduction in vertigo induced by rotation at a constant velocity of $138^\circ/\text{s}$ about a vertical axis. Motion sickness and disorientation are associated with misalignment of the axis of eye velocity from gravity. This misalignment may provide a reference for assessing both subjective orientation and the potential for producing motion sickness.

The studies at Brandeis seek to distinguish vestibular and motor factors in AG side effects. Motor factors can be isolated by studying subjects pointing to targets without moving their head during constant velocity rotation. Initially, reaching paths and endpoints are deviated in the direction of the transient lateral Coriolis forces generated. With practice, subjects soon move in straighter paths and land on target once more. If sight of the arm is permitted, adaptation is more rapid than in darkness. Arm movement trajectory and endpoint deviations are proportional to Coriolis force magnitude over a range of rotation speeds from five to 20 rpm, and there is rapid, complete motor adaptation at all speeds. Coriolis force perturbations of motor control are also characteristic of unrestrained head movements in a rotating room, which involved both vestibular and motor factors.. The results indicate that motor adaptation to high rotation rates is possible. An effective countermeasure against the side effects of head movements must act upon both the vestibular and neuromotor systems.

**NSBRI RESEARCH PROGRAM
NUTRITION, PHYSICAL FITNESS AND REHABILITATION**

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RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Marco E. Cabrera, Ph.D.
ORGANIZATION:	Case Western Reserve University
PROJECT TITLE:	Metabolic Adaptations of Skeletal Muscle to Training/Detraining: A Systems Model

Project Executive Summary

Space travel (*detraining*) has detrimental effects on skeletal muscle structure, metabolism, and function, including reductions in muscle size, strength, and endurance. Exercise (*training*) in space can counteract some of these deleterious effects. Indeed, experimental studies are still being conducted to determine both the cause of muscle deterioration and the exercise training programs needed to counteract the detrimental effects of long-duration space travel on muscle function. In addition to obtaining relevant metabolic data from space and ground-based studies, physiologically-based computational models of human function are needed to integrate cellular to whole-body data and to provide a framework for quantitative understanding of the skeletal muscle metabolic responses to exercise in the trained and detrained states.

The specific aims of this project are:

1. To identify the metabolic adaptations to training and detraining in order to develop databases containing (a) information on the structural, metabolic, and functional adaptations of skeletal muscle to microgravity and exercise training and (b) the underlying biochemical mechanisms mediating these adaptations.
2. To develop mathematical models of intermediary metabolism in skeletal muscle that account for the effects of training and detraining.
3. To investigate the relative significance of model parameters affected by training or detraining on work capacity and efficiency.
4. To simulate the effects on skeletal muscle intermediary metabolism and energetics of space flight and exercise in space, to quantitatively test the hypotheses that after a period of space travel or exercise training, the observed changes in the rates of carbohydrate and fatty acid oxidation in skeletal muscle are a result of (a) a partial conversion of slow-twitch to fast-twitch fibers and (b) alterations in glycolytic and oxidative enzymes.

During the second year of the project we continued the development and implementation of a computational model of skeletal muscle metabolism that integrates cellular, tissue, and whole body data and that incorporates specific parameters which have been identified as playing a major role in the responses to training and detraining such as muscle mass and enzyme activities. Computer simulations of responses to moderate exercise were performed on three muscle models representing different states: (a) normal sedentary subject, (b) trained subject, and (c) detrained subject. Then, we contrasted the exercise responses resulting from the model of a "trained muscle" to those from the model of a "detrained muscle."

We also continued collaborating with other NSBRI investigators in the development of:

- a) A comprehensive model of the human body and its responses to exercise, in collaboration with Dr. Martin Kushmerick (Muscle Alterations Team) and Dr. James Coolahan (Cardiovascular Alterations Team), and
- b) Methods to evaluate the effectiveness of exercise training programs in space, in collaboration with Dr. Babs Soller (Smart Medical Systems Team).

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Joanne R. Lupton, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Nutritional Countermeasures to Radiation Exposure

Project Executive Summary

Original Aims

The overall goal of this research program is to develop nutrition countermeasures to radiation-induced colon tumorigenesis, using male Sprague Dawley rats as a model system. Superimposed on the background of irradiation with Fe-ions or no irradiation is the injection of a known colon specific carcinogen, azoxymethane (AOM) in order to simulate the potential exposure to environmental contaminants. The diet interventions to be tested are combinations of a lipid component (fish oil vs corn oil) and a fiber component (pectin vs cellulose). At the end of the three year period we will know: (1) if radiation exposure synergistically enhances colon tumor induction by AOM; (2) which diet combination(s) are protective against colon cancer and if this effect is due to less DNA damage, greater removal of DNA-adducted cells by apoptosis or greater repair of DNA-adducted cells; (3) if short term studies (e.g. initiation or aberrant crypt formation) are predictive of later tumor development; and (4) if noninvasive technology can be used to detect specific mRNAs that are predictive for radiation exposure and/or response to that exposure, which would have later application to humans.

Key Findings

The preliminary project results demonstrated that our selected radiation dose and sampling times were appropriate for the proposed experimental design. We discovered that prior exposure to radiation before exposure to a chemical carcinogen increased the severity of the preneoplastic lesions formed during colon tumorigenesis. Expression of genes associated with response to carcinogen exposure, as well as control of cell cycle kinetics is influenced by radiation exposure, above and beyond that observed with the chemical carcinogen. The dietary manipulations demonstrate that fermentable fiber and n-3 fatty acids are able to reduce the formation of preneoplastic lesions of colon cancer. Once the project is completed and we have all the results available, there should be an effect of diet on ameliorating colon cancer development.

Impact of Findings on Project Goals

The initial findings from our experiment indicate diet is capable of reducing the preneoplastic lesions that lead to colon cancer. Therefore, diet may serve as a viable countermeasure to help maintain astronaut health.

Proposed Research Plan for the Coming Year

In addition to completing the remaining work on samples collected during the first two years, the experiment will be conducted this year on the last half of the rats treated only with the carcinogen.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Ronenn Roubenoff, M.D.
ORGANIZATION:	Tufts University
PROJECT TITLE:	Timed Feeding and Resistance Training to Prevent Muscle Atrophy

Project Executive Summary

Resistance training (RT) is one modality that offers the hope of mitigating or reversing muscle loss induced by weightlessness. Recent studies suggest that the timing of feedings around a bout of RT may maximize the effect of exercise on muscle protein balance. We therefore propose to test whether timed feedings, given before RT sessions, is superior to RT alone as a countermeasure against muscle atrophy induced by bed rest. This proposal is directly applicable to the NSBRI Nutrition, Exercise and Rehabilitation Team's first Focused Research Question, on Exercise Countermeasures. We hypothesize that:

1. The combination of timed feeding of an amino acid (AA) and sucrose mixture with RT (TFRT) is more effective than RT or feeding alone as a countermeasure against bed-rest induced muscle atrophy.
2. TFRT accelerates recovery from 28 days of bed rest compared to either RT alone or feeding alone over 14 days following strict bed rest.
3. TFRT works at least in part by modulating muscle levels of anabolic growth factors and cytokines IGF-1, TGF- β , IL-15; and catabolic cytokines IL-1 β , IL-6, TNF- α .

We will study healthy adult men aged 30-55, with BMI 23-28 kg/m², who will undergo 28 days of strict bed rest followed by 14 days of standardized weight-bearing physical activity on a metabolic ward. Subjects will eat 15 percent less than their energy requirements to replicate space-based anorexia. Subjects will be randomly assigned to one of three groups for the six-week study period: a) a sucrose/AA supplement given every other day (timed feedings given every 48 hours [TF group], which will be the control group for the study); b) RT every other day (RT group), with the supplement provided 24 hours after the RT to insure that the three groups are isoenergetic and isonitrogenous; c) RT every other day with the supplement given five minutes before the RT session (TFRT group). Outcomes will include body composition measured at multiple levels: total body potassium [TBK]; body fat and bone by dual-energy x-ray absorptiometry [DEXA]; bone turnover using urinary markers; regional muscle (mid-thigh muscle) computerized tomography [CT]; and cellular level (histochemistry of muscle fiber area and type distribution). We will also examine muscle strength and functional performance, and muscle gene expression of anabolic (IGF-1, TGF- β , IL-15) and catabolic (IL-1 β , TNF- α , IL-6, myostatin [GDF-8]) signals in muscles.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Brian W. Tobin, Ph.D.
ORGANIZATION:	Mercer University School of Medicine
PROJECT TITLE:	Nutritional Modulation of Pancreatic Endocrine Function in Microgravity

Project Executive Summary

Our specific aims in this study are to: 1) assess the effect of a microgravity model cell culture on basal amino acid requirements and endocrine secretory function in human islets of Langerhans, and 2) determine human islet endocrine function while testing amino acid countermeasures in the microgravity model.

Ground based and in-flight investigations illustrate changes in insulin, glucose, and amino acid metabolism in spaceflight. These observations may relate to altered pancreatic endocrine function which is insufficient to meet the needs of microgravity induced insulin resistance, and altered amino acid metabolism. The changes observed include decreased glucose tolerance, increased circulating insulin, and increased reliance upon glucose in muscles. The metabolic milieu resembles an insulin resistant syndrome, accompanied by a compensatory increase in pancreatic insulin secretion. However, the increase in insulin secretion is insufficient to ameliorate muscle atrophy. The increased insulin secretion is well correlated to muscle atrophy in spaceflight. The influence of these changes upon the loss of muscle mass and general endocrine metabolic state are not well established, however. Countermeasures which could modulate insulin and glucagon secretion in a compensatory manner to overcome insulin resistance and promote amino acid uptake by peripheral musculature might decrease muscle atrophy and reduce injury following re-adaptation to unit gravity.

We hypothesize that human pancreatic islets of Langerhans have an increased requirement for amino acids in microgravity. We further hypothesize, that supplementation with specific additional amino acids will augment, enhance and normalize insulin secretion, when spaceflight paradigm stressors known to decrease insulin secretion, are applied.

It is anticipated that these studies will further refine our understanding of human pancreatic amino acid requirements and endocrine regulation: phenomenon which may be limiting to extended-duration spaceflight missions. These studies will test countermeasures to augment pancreatic endocrine function, while considering both insulin and glucagon production in a way that will involve supplementation of diet with additional amino acids. These measures are ultimately aimed at improving spaceflight induced muscle atrophy, and ameliorating current re-adaptation constraints.

Key Findings

We have accomplished a part of Specific Aim 1: “To assess the effect of a microgravity model cell culture on basal amino acid requirements and endocrine secretory function in human islets of Langerhans.”

Our results of experiments conducted this year in which human pancreatic islets of Langerhans were cultured in the HARV bioreactor and contrasted to controls show reveals the following key findings:

1. There is a tendency towards less glucose utilization in HARV-cultured islets of Langerhans
2. There is a tendency towards enhanced insulin secretion in islets maintained in the HARV,
3. We observed differential alterations in the pattern of amino acid utilization in the HARV,
4. Islet TNF production favors greater activity in the HARV cultures.

Impact of these findings on the hypotheses or requirements (technology), objectives and specific aims of the original proposal:

Observation A: The tendency towards decreased glucose utilization in HARV-cultured human islets of Langerhans, supports the hypothesis that microgravity is associated with a sub-clinical diabetogenic state. The observation of lesser glucose utilization in human islets is consistent with observations of rat islets cultured in the HARV system when contrasted to controls.

Observation B: The increased insulin secretion in the pancreatic islets cultured in the HARV suggests that islets are responding to some stimuli similar to that observed in insulin resistant states. It is well established that even in the face of severe insulin resistance, and decreased uptake of amino acids by muscle in diabetic individuals, the output of insulin by the pancreas is dramatically increased. This scenario is consistent with the observations in human pancreatic islets of Langerhans in the HARV microgravity model system.

Observation C. The differential pattern of amino acid utilization is consistent with the hypothesis that microgravity causes alterations in the pattern of metabolic substrate utilization. This is consistent with published data, and supports the hypothesis that the peripheral tissues are not the only sites of altered amino acid metabolism. The pancreatic islets of Langerhans also appear to be altered in their patterns of metabolite use when cultured in a microgravity model system.

Observation D: The greater TNF production in pancreatic islets of Langerhans supports the hypotheses that insulin secretion is suppressed from reaching an adequate level sufficient to overcome peripheral insulin resistance in muscle tissue. That TNF can suppress insulin action is well established. That TNF is secreted by pancreatic islets of Langerhans was previously reported by our laboratory. Given that TNF in HARV cultures is increased, this scenario suggests that even in the face of a need for increased insulin secretion to overcome insulin resistance in muscle, that TNF may be suppressing a maximal beneficial response in the islets of Langerhans

Proposed research plan for the coming year

In the coming year we plan to accomplish a comprehensive analysis of the effects of: 1) microgravity simulation, 2) LPS, 3) epinephrine, 4) cortisol, and 5) amino acid administration, upon endocrine and cytokine function as well as the nutritional utilization of glucose, amino acids, and fatty acids.

We will accomplish this by carrying out studies according to the following description.

1) Approximately 24,000 human cadaveric pancreatic islets of Langerhans are isolated and purified by collaborator JRT Lakey, PhD, at the University of Alberta and shipped in Medium-199 to Mercer University School of Medicine (MUSM).

2) At MUSM, human islets of Langerhans are prepared for bioreactor culturing by collaborator SK Leeper-Woodford, BW Tobin, PhD, and PN Uchakin, with the technical expertise of Cynthia

Bruin, and are allocated into 10 ml disposable High Aspect Ratio Vessels (HARV) or standard 10 ml cell culture plates.

3) The 24,000 human islets of Langerhans are divided into independent variable group.

EFFECTS OF STRESSORS

HARV w/ LPS	HARV w/ Cortisol	HARV w/ Epinephrine	HARV w/ no treatment
Plate w/ LPS	Plate w/ Cortisol	Plate w/ Epinephrine	Plate w/ no treatment

EFFECTS OF AMINO ACIDS

HARV with Arginine	Plate with Arginine
HARV with WOLFE Amino Acids	Plate with WOLF Amino Acids

4) The islets are cultured for 48 hours and samples are taken at 0, 3, 6, 12, 24 and 48 hours for analysis of metabolites by our collaborative group. e) Aliquots of islet medium are frozen at -70° C and are subsequently shipped to the following collaborators for dependent variable analysis.

Dr Uchakin (MUSM): glucose, lactate, insulin, glucagons

Dr Leeper-Woodford (MUSM): TNF-alpha, Il-1, Il-6, NF kappa beta

Dr. Walzem (TAMU): fatty acids, lipids,

Dr. Smith (NASA-JSC): amino acids, nitrogenous compounds

5) Data are returned from the collaborators to MUSM and are analyzed by Drs. Uchakin, Tobin and Leeper-Woodford. All members of the PI and Co-I and add-on project team confer on data analysis and interpretation. All project team members are eligible for inclusion as authors on any or all abstracts, presentations, or manuscripts resulting from these studies. NSBRI is properly acknowledged in all presentations and publications.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Robert R. Wolfe, Ph.D.
ORGANIZATION:	The University of Texas Medical Branch - Galveston
PROJECT TITLE:	Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress

Project Executive Summary

Specific Aims

Prolonged space flight causes a loss of skeletal muscle mass that is detrimental to physical function, and amelioration of this response is essential for successful prolonged missions. There are two components of the loss of muscle mass in space flight. Prolonged muscular inactivity causes a reduction in protein synthesis, while at the same time stress (mediated by moderate hypercortisolemia) accelerates the rate of muscle protein breakdown, at least insofar as it relates to the rate of synthesis. Our previous work in normal volunteers has shown that a supplement containing a mixture of essential amino acids and carbohydrate stimulates muscle protein synthesis. Further, whereas ingested amino acids normally do not affect the rate of muscle protein breakdown, they limit the accelerated rate of breakdown that occurs in stress states, such as in severely burned patients. Consequently, we anticipate a mixture of essential amino acids (15g) and carbohydrate (30g) given as a supplement three times per day will limit the loss of muscle, and in turn muscle function, during our model of space flight, which is prolonged bed rest + hypercortisolemia. We are testing the hypothesis that essential amino acid/carbohydrate supplementation will ameliorate the loss of lean body mass and muscle function that occur after 28 days of bed rest, while improving nitrogen balance over the duration of the experiment. Further, we have quantified muscle amino acid and protein kinetics at the beginning and the end of bed rest in order to gain insight into the mechanisms responsible for the loss of muscle mass in untreated subjects, as well as into the mechanisms by which supplementation serves to decrease muscle catabolism. Specifically, we have determined muscle amino acid and protein kinetics over 24 hour periods before and at the end of bed rest in order to address the following hypotheses:

1. The normal anabolic response of muscle to a meal diminishes with prolonged inactivity and stress.
2. An amino acid/carbohydrate supplement will stimulate net muscle protein synthesis over the one-hour immediately following ingestion.
3. The normal anabolic effect of meals will not be affected by prior ingestion of a supplement.
4. The post-absorptive nadir in net muscle protein synthesis will be no greater in subjects receiving supplementation than in control subjects.

Key Findings

We have completed the study of seven subjects on the essential amino acid/carbohydrate supplement (EAA group) and six subjects receiving placebo (Placebo group). The findings indicate that supplementation with EAA maintains lean body mass (LBM) throughout 28 days of bed rest, while the placebo group experiences a loss of LBM. The EAA supplement maintains LBM by stimulating net muscle protein synthesis to a much greater extent than meal ingestion alone. Although this stimulation is diminished with increased inactivity, the net gain in muscle protein is still significantly greater than that produced by meals alone. In other words, even

though the anabolic response to the EAA supplement decreases after 28 days of bed rest, it is still capable of producing a significant increase in net muscle protein synthesis.

Though EAA supplementation is capable of maintaining LBM, it does not maintain muscle strength. Measures of leg muscle strength decline after 28 days of bed rest despite the preservation of leg lean mass. These findings demonstrate that the maintenance of LBM alone is insufficient in terms of muscle function. Apparently, some neuromuscular component is also required to preserve muscle strength and function.

Our findings also demonstrate that the EAA supplement is capable of stimulating net protein synthesis when given in a stressed state, as simulated by cortisol infusion. The presence of elevated blood cortisol induces a loss of muscle protein even when a meal is given. Though the EAA supplement can slow this loss, it only does so temporarily, such that within one to two hours after the supplement, the muscle protein balance is again catabolic. After 28 days of inactivity, the response to a meal during elevated cortisol is further diminished, such that the muscle is dramatically catabolic. The EAA supplement is not capable of eliciting an anabolic response in the muscle after 28 days of bed rest. On the contrary, when the supplement is given without the presence of cortisol, the net effect is muscle anabolism over the study time period.

Impact of Findings

Our findings demonstrate that the anabolic response to a meal diminishes with prolonged inactivity and a stress challenge. The stimulation of net muscle protein synthesis immediately following each EAA supplement translates to a maintenance of muscle protein over a 24 hour period, and in turn, over the 28 days of bed rest. However, the maintenance of LBM does not translate to maintenance of muscle strength. The interaction of inactivity and stress exacerbates the ineffectiveness of ordinary meals. Though the EAA supplement can offset muscle catabolism during the stress state, the response is transient and incapable of ameliorating the overall loss of muscle protein. Taken together, these findings indicate that a nutritional supplement alone can reduce the muscle atrophy associated with space flight. However, whereas muscle mass can be maintained with a specified nutritional intervention, other modalities are required to preserve muscle function.

Proposed Research Plan

We are currently studying the effects of chronically elevated cortisol throughout 28 days of bed rest on muscle protein, LBM, and muscle function. As of this writing, we have completed 1 subject and are currently studying two more. This investigation will determine the interaction of chronically elevated cortisol and muscular inactivity on the loss of LBM and muscle function. This investigation will help determine an optimal operational countermeasure that can be economically (in terms of crew time and payload) utilized to ameliorate muscle loss during prolonged space flight.

**NSBRI RESEARCH PROGRAM
RADIATION EFFECTS**

Team Leader:	Dicello, J. F.	Hopkins/SOM	
Associate Team Leaders:	Kennedy, A. R. Vazquez, M. E.	Penn Brookhaven	
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Anderson, C. W.	CO-I Brookhaven		

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Polly Y. Chang, Ph.D.
ORGANIZATION:	SRI International
PROJECT TITLE:	Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice

Project Executive Summary

Evaluations of risks involving alterations in the genome using whole animal systems are essential to missions in space. The *lacZ* transgenic mouse model is the only system available, to date, for the assessment of alterations in the genome in every tissue of the animal. In this model system, every cell of the animal contains multiple copies of an integrated but inert target transgene. Radiation-induced mutations can be measured and specific genetic alterations characterized using established protocols. Genetic alterations in tissues that are of high priority in NASA's Strategic Program Plans, but are not accessible using conventional techniques, e.g., the central nervous system, can be evaluated using this model system. In addition to measuring short (one week) and long term (up to 16 weeks after treatment) mutagenicity in the reporter transgene, concomitant evaluation of the clastogenic potential of highly charged and energetic (HZE) particle radiation can also be done using the same experimental animals. Some of these include examining radiation responses in the hematopoietic system by enumerating micronuclei (MN) in peripheral blood, evaluating chromosomal damage in either circulating or bone marrow lymphocytes by using fluorescence *in situ* hybridization (FISH) techniques, and induced gene expressions in tissues by using RT-PCR. Specifically, our research aims for this project include the use of *lacZ* transgenic mice to characterize the dose- and time-dependent radiation-induced responses in *lacZ* transgenic mice after high Linear Energy Transfer (LET) iron particle beams generated at Brookhaven National Laboratory and low LET proton irradiation at the Loma Linda University Medical Accelerator. We will measure the initial effects and long-term residual consequences of radiation exposure in tissues that are of high priority to NSBRI and NASA, namely the brain (CNS) and compare these responses to another tissue such as the spleen that is known to be a highly proliferative tissue with stem cell populations. We hypothesize that the *lacZ* mutation frequency (MF) in individual tissues will increase as a function of dose for each tissue, that this response is LET dependent, but the level of induction of MF is dependent on the specific tissues analyzed. Micronuclei (MN) in peripheral blood have been used extensively as a biomarker to evaluate radiation toxicity in the human population. We aim to examine radiation responses in the hematopoietic and lymphatic system in the same experimental animals. We expect that the level of genetic damage as well as the kinetics of removal of aberrant cells and the spectrum of chromosome aberrations is dose dependent.

Variations in genetic background have been shown to impact an individual's sensitivity to radiation exposure. The tumor suppressor *p53* gene function has been shown to be radiation responsive and very important in the regulation of cell growth, proliferation, differentiation, and apoptotic signaling pathways in many tissues. We cross-bred the C57 *lacZ* transgenic mice that are wild type for *p53* (*p53*^{+/+}) with *p53* nullizygous (*p53*^{-/-}) mice to establish breeding colonies of transgenic mice, all possessing the *lacZ* transgene and are either hemizygous or nullizygous for *p53*. These animals will be used to assess tissue-specific *p53*-dependent (or -independent) molecular and genetic mechanisms in radiation-induced damage resulting from exposure to particle beams in the energy range relevant to space radiation. Specifically, animals with different *p53* genetic backgrounds will be exposed to a range of doses of either iron particles or

proton radiation and tissue-specific radiation responses using the same endpoints as mentioned in the previous section will be evaluated. Results from these studies will reveal the impact of variation of genetic background to an individual's sensitivity to radiation exposure of different LETs.

Research activities for our second funded year include characterizing the dose and temporal dependence of iron-particle induced genetic damage in the *lacZ* transgenic mice and continued efforts to analyze samples archived from animals exposed to proton exposure.

We have previously reported that protons are effective in inducing dose-dependent increases in the levels of micronucleus in immature reticulocytes (MN-RET), a well accepted marker for genetic toxicity, in peripheral blood of mice. This effect appeared to saturate at higher doses. Animals exposed to an acute 4 Gy proton dose showed symptoms of transient systemic toxicity as evidenced by a dramatic drop in total reticulocyte counts shortly after radiation treatment. The percentage of MN-RET also varied as a function of time (24 - 120 hours) after exposure and in most animals, complete recovery was observed at one week after exposure. New evidence gathered during the Spring 2002 showed that the levels of MN-RET in peripheral blood in animals exposed to a range of 1 GeV/amu Iron ions were significantly higher than those from the controls at doses ≥ 0.5 Gy as early as 17 hours after exposure. The MN-RET levels returned to control levels for lower doses within three days, but were delayed up to one week post exposure for animals exposed to higher doses. Fluence-based analysis of the MN-RET data revealed that the relative effectiveness of iron ions in inducing peripheral blood MN-RET appears to be similar to those of protons at this time point, suggesting that the RBE for MN-RET may be approximately one for this end point.

We have completed the analysis of proton radiation induced changes in *lacZ* transgene in the brain and spleen tissues at one, eight and 16 weeks after exposure. New information generated from spleen tissues at 16 weeks post irradiation demonstrated that mutant frequencies (MF) in the transgene, although still higher than spontaneous MF in the control animals, was reduced when compared to the eight week data for doses ≥ 0.5 Gy, suggesting that either the radiation induced damage to the genome was repaired or that the heavily damaged cells were eliminated from the cell population. Mutation results from the 16 week 0.1Gy irradiated spleen tissues were interesting in that the MF was persistently higher than their age-matched sham-treated controls. These results suggest that tissue recovery mechanisms after low dose exposures may be different from those that were exposed to high radiation doses and warrants further investigation.

LacZ MF in the brain tissues after proton exposure showed that proton exposures induced significantly higher transgene MF as early as one week after radiation exposure. Such elevated levels of MF persists for doses ≥ 0.5 Gy doses at the later eight and 16 weeks post irradiation when compared to the parallel sham-treated control animals. Although the overall MF in this tissue is lower than those obtained from the spleen at each of the time points, elevated MF at late time points in this tissue may be of particular concern to the Space Radiation Health community in terms of long term risk for the CNS tissue. These *in vivo* observations may provide important information regarding late tissue-specific effects that are not readily available in *in vitro* cell model systems.

Transgene MF in the brain and spleen tissues were measured after animals were exposed to an acute range of high LET Iron particle radiation. Preliminary results show that indeed, this *in vivo* model system is sensitive to the detection of HZE-induced genetic damage in tissues. In the brain, up to > 2 fold higher than spontaneous MF was observed in samples harvested from

animals at eight weeks after they were exposed to a single high dose of 2 Gy. In the spleen tissue, this effect was less pronounced, with the increase in MF reaching a plateau at doses > 1 Gy. When compared with the proton results, the current findings suggest that induction of *lacZ* MF in tissues is not only tissue specific, temporally regulated but also dependent on LET of the incident radiation beam.

To address NASA's interests in examining potential countermeasures that will protect astronauts in long term space missions, we had initially proposed examining the effects of the pro-inflammatory cytokine IL-1, known to be a low LET radioprotectant, to alter the level of radiation-induced genetic damage after proton radiation. However, due to the reviewer's concerns regarding the use of this cytokine, we have redirected our research in this aim to study the use of currently available passive shieldings relevant to space travels. Animals were exposed to aluminum - or polyethylene-modified protons or iron irradiations and biological responses, including cytogenetic measurements as well as MF in the *lacZ* transgene were measured as a function of time and dose. Our hypothesis is that the profile of beam energy and charge will be modified as particle beams traverse through different materials. We have results showing that the MN-RET yield in peripheral blood, *lacZ* MF in the brain and spleen after 2 Gy of protons with or without aluminum shielding were the same, suggesting that aluminum did not afford any protection for the animals at this dose level, possibly due to effects of fragmentation of the proton beam in the metal. In the Spring 2002 BNL experiment, animals were exposed to a low (0.1Gy) or high (1 Gy) dose of 1 GeV/amu Iron particles with or without 10 or 15 cm polyethylene. Preliminary MN-RET results from these studies show no difference in the percentage MN-RET in peripheral blood between the shielded and their unshielded counterparts. These results are consistent with published observations made with shielding studies on human lymphocytes.

The construction of a new dedicated beam line at Brookhaven National Laboratory is now complete and we have obtained approved beam time for 1 GeV/amu Iron ions during the upcoming NSRL-1 run (October - November 2003). During this run, we aim to expose *lacZ* animals that are either *p53* hemizygous or nullizygous to a range of Iron doses (0.1 – 2 Gy) and monitor cytogenetic damage and recovery in these animals in both the circulating reticulocytes as well as the bone marrow compartments. Animals will also be housed up to 16 weeks after exposure so that late tissue responses can be measured using the transgenic mutagenesis assays. Our results obtained from these studies will be compared to the results we already have on hand for the *p53* wild type animals to determine the impact of *p53* genetic status on radiation sensitivity.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	John F. Dicello, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Radiation Effects Core Project: In Vivo and In Vitro Studies

Project Executive Summary

The risks of cancer to personnel in space from the naturally occurring radiations are generally considered to be one of the most serious biomedical limitations associated with long-term human space missions, as noted in two recent reports of the National Research Council/National Academy of Sciences. The paramount goals of the Radiation Effects Team for the National Space Biomedical Research Institute are: to determine carcinogenic consequences of radiations in space in an appropriate model; to develop effective physical and pharmaceutical countermeasures; and to study ways to reduce the risks of cancer and other diseases associated with such exposures.

During interplanetary missions, personnel in space will be exposed to galactic cosmic rays, including high-energy protons and energetic ions. (Ions with high energy, E, and atomic numbers, Z, greater than one are usually called HZE particles.) In addition, solar events will produce radiation fields of high intensity for short but irregular durations. The level of intensity of these radiations is considerably higher than that on Earth's surface, and the biological risks for carcinogenesis to astronauts are consequently elevated. Our group is examining the risk of cancers in model systems resulting from their low-dose exposures to photons, protons, and iron by using ground-based accelerators, which are capable of producing beams of such particles at energies comparable to those encountered in space. We have successfully conducted a series of experiments using a 1-GeV iron beam at the Brookhaven National Laboratory and 250-MeV protons at Loma Linda University Medical Center's proton synchrotron facility. As part of these studies, we have been collaborating with a companion project (David Huso, Principal Investigator) that is investigating the potential for anti-estrogen-based pharmaceuticals to reduce the risk of cancer after irradiation at the level of doses and for particle types expected in space. The hypothesis is that carcinogenesis in in-vivo models can be used to extrapolate to risk in humans and the risk of hormone-stimulated cancers such as breast cancer can be reduced by the subsequent administration of appropriate drugs after exposures to protons and densely ionizing radiations such as energetic heavy ions and neutrons. Additionally, the hypothesis is that the precursors of cancer can be altered at the promotion and progression stages of the diseases rather than the initiation. If this latter hypothesis is correct, it could reduce or eliminate the need for administering drugs in anticipation of significant exposures.

Theoretical studies carried out in a collaboration between scientists at NASA's Johnson Space Center and Johns Hopkins University are providing methods and predictions which are being used to assess the levels of radiation risks to be encountered, and to evaluate appropriate strategies for countermeasures. Continued collection and analysis of data from this project over the next three years will further enhance the precision of our estimates of biologic response and reduce the large uncertainties associated with previous assessments of risks for activities in space.

The research has consisted of four successive series beginning with a feasibility study to develop the logistics and infrastructure for these types of studies and followed by three studies to investigate the incidence of mammary carcinomas and the change in risk with the subsequent administration of Tamoxifen. The final three studies are still in progress, but the first one has been successfully completed, and the data are being examined and analyzed. The initial results provide some of the first in-vivo data for the risk of cancer from both energetic protons and heavy ions. Initial results from the companion project indicate validation of the hypothesis that the risk can be reduced by subsequent pharmaceutical intervention with drugs.

Although the work in this project is primarily directed toward risks associated with space travel, the problem of protracted exposures to low-levels of radiation is one of national interest in our energy, defense, and homeland security programs, and the present results suggest new paradigms for addressing such risks.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	David L. Huso, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Chemoprevention and Radiation-Induced Neoplasms

Project Executive Summary

Chemoprevention is a pharmaceutical approach to arresting or reversing the process of carcinogenesis during cancer's typically prolonged latent period (often 20 years or more) before invasion or metastasis occurs. Surging scientific and public interest in applying chemoprevention strategies to people in the general population that have been identified to carry even slight increases in the risk of developing cancer (e.g. genetic risk) is fueling the identification of exciting new chemopreventive agents. Some now argue that future development of chemopreventive agents offers greater potential for the long-term control of cancer than the much more widely studied and aggressively pursued chemotherapy agents.

The major long-term risk associated with radiation exposure received during space travel is predicted to be radiation-induced cancer. The cancer-causing effects of low-LET radiations such as x-rays, g-rays, or electrons, typical of environmental earth exposures, have been relatively well-established. However, radiation likely to be encountered in space includes mainly heavy ions and protons along with their secondaries. Much less is known about the biology and risks associated with these types of radiation. The doses of radiation likely to be received even for long missions are probably low, but cover a broad range and are very unpredictable due to solar events. Like other types of radiation, the increased cancer risk associated with proton and heavy ion exposure is troubling because many radiation-induced cancers do not appear until later in life. Therefore, a large amount of uncertainty exists in how best to assess and manage the radiation risks associated with space travel.

Two high priorities in preparation for long term missions are 1) providing a better understanding of both the short-term and long-term carcinogenic risks of heavy ion or proton radiation and 2) developing pharmaceutical countermeasures to mitigate the carcinogenic risk associated with low-dose and mid-dose exposures to these types of radiation. Currently there are 3 cancer chemopreventive strategies that have clearly proven efficacy in preventing human familial and sporadic cancers 1) selective estrogen receptor modulators for prevention of breast cancer, 2) NSAID's (nonsteroidal antiinflammatory drugs) which may prevent a variety of cancers, and 3) retinoids for certain epithelial cancers. As countermeasures to the cancer risk associated with space travel, these chemopreventive approaches offer a particularly promising approach for countermeasure investigation because of: 1) these compounds are currently being used as preventives for human cancers although they are untested against proton or heavy ion-induced cancer, 2) there are difficulties associated with absolutely blocking radiation-induced mutagenic damage to DNA during prolonged space travel, either with shielding or pharmaceuticals, and 3) the prolonged latency period of most radiation-induced cancers (especially at low doses) offers a prolonged time period to administer chemopreventives. This is important since the latency period is the time when the most successful chemopreventives exert their effects. For most cancers, compounds that modulate the regulation of cell growth and apoptosis (rather than blocking mutagenic damage to DNA) have to date shown particular promise in preventing overt cancer from developing in susceptible organs.

Organs are not equally sensitive to the carcinogenic effects of radiation. Tissues that appear to be at higher risk for developing radiation-induced neoplasms include the female breast, the gastrointestinal tract (colorectal cancer), the thyroid, the bone marrow/lymphoid system (leukemia), and the lung. Women have an increasing role in the space program. The female breast is particularly sensitive to the carcinogenic effects of radiation and therefore a relevant tissue in which to study chemoprevention of radiation-induced cancer. Chemoprevention of radiation-induced cancer in this sensitive target organ provides an excellent system in which to initially gain insights into the chemoprevention of radiation-induced cancer in general.

Over the past few years, tamoxifen has not only emerged as an effective chemopreventive against breast cancer, but it has also become the most widely prescribed anticancer drug in the world. It is a prototype of the group of pharmaceuticals called selective estrogen receptor modulators. Tamoxifen had been used for over 25 years for breast cancer treatment prior to its application as a chemopreventive. This level of acceptance for use in humans along with its proven chemopreventive efficacy against sporadic breast cancer provides a strong rationale for investigating its safety and efficacy against breast tumors induced by heavy ions and protons. As a potential countermeasure to the risks associated with prolonged space missions, the tamoxifen family of compounds have outstanding potential with a high level of readiness.

The class of compounds that includes tamoxifen, the selective estrogen receptor modulators (SERM's), are thought to have outstanding potential both in estrogen replacement therapy and as chemopreventive agents. Burgeoning research and development of new SERM compounds has led to many new and improved SERM's undergoing trials. Tamoxifen, however, remains the prototype SERM for breast cancer chemoprevention. Newer SERM's will hopefully further improve on tamoxifen's effects while reducing its side effects. SERM's are ligands for the estrogen receptor (ER) and modify carcinogenesis in breast epithelial cells by antagonizing ER signaling. However, in other tissues SERM's can act as partial ER agonists and promote the beneficial effects of estrogens in, for example, the skeletal and cardiovascular systems. Interestingly, tamoxifen may also affect carcinogenesis in a number of organ systems by disrupting apoptosis regulation in proliferating cells. In spite of the widespread use of tamoxifen, very little is known about its lifetime effectiveness against radiation-induced neoplasms-particularly those induced by radiation likely to be encountered in space such as protons and heavy ions.

In vivo studies provide a powerful means for directly evaluating the effectiveness of particularly promising chemopreventives against cancers that may occur following radiation exposure. The rat mammary tumor model has been used extensively to analyze the carcinogenic effects of both chemical xenobiotics and physical agents. The Sprague Dawley rat mammary tumor model is particularly well-suited for studies in the low dose range because this model is prone to develop induced mammary neoplasms early in life. Previous studies using the Sprague Dawley model have shown that sublethal doses of radiation (x-rays, gamma rays, neutrons-not particularly relevant to space travel) induce mammary tumors, often within one year, and with a linear dose-effect relationship. Thus the Sprague Dawley rat mammary carcinogenesis model not only closely resembles human breast cancer biologically, but it also is a highly sensitive model in which to examine the effects of radiation exposure and for testing pharmaceutical countermeasures against radiation effects. Our initial studies have focused on the effects of whole body, low level heavy ion and proton radiation along with chemoprevention of similarly induced mammary tumors using the female Sprague-Dawley rat mammary tumor model. The well-studied, widely prescribed, prototype SERM, tamoxifen has been effectively and safely used in humans for chemotherapy for almost two decades. These advantages, along with an understanding of its

molecular mechanism of action, suggest it would be an excellent candidate for successful long-term chemoprevention of specific proton and heavy ion-induced cancers. The prospect for successful long-term chemoprevention of this potentially important, late-appearing cancer relevant to space radiation exposure is indeed an exciting prospect.

Hypothesis and Aims:

There is an uncertain, but serious risk of cancer potentially associated with prolonged space travel. These risks cannot be addressed with shielding alone. Our first hypothesis is that modeling these risks can remove much of the uncertainty and would allow better management of some of the radiation-risks associated with prolonged space missions. Our second hypothesis is that the increased cancer risk that may be associated with radiation in the space environment can be mitigated by chemopreventive countermeasures implemented during the long cancer latency period that follows radiation exposure. The cancer causing effects of radiation as well as the safety and efficacy of chemopreventives have not been determined under conditions relevant to space. Animal models provide the best tools to test these hypotheses in relevant settings and should provide important insights into the chemoprevention of breast cancer in the general population.

Specific Aims:

- 1) To determine the relative risks associated with exposure to the types of radiation encountered in space using a sensitive *in vivo* model of radiation-induced cancer.
- 2) To determine if pharmaceutical cancer chemopreventives could provide a safe and effective countermeasure approach to mitigate the cancer risk that may be associated with exposure to the types of radiation likely to be encountered in space.

Key findings:

Although our studies are not complete, *preliminary trends in our tamoxifen studies have pointed to a proof of principle for a strategy in which chemopreventive agents could play an important role in preventing breast cancer following exposure to radiation during space travel.* Confirmation of these trends is still pending the completion of these studies. Since Dr. Huso took over as PI of the chemoprevention studies, considerable progress has been made in this area. Since cancer chemoprevention in general is still in its infancy as an emerging field, chemoprevention based on new targets and emerging compounds, hold considerable promise for continued improvement of strategies to effectively mitigate risks associated with radiation and other predisposing factors for cancers. During the coming year we plan to complete our studies on tamoxifen chemoprevention of radiation-induced cancer and analyze in detail the overall findings. The data should give new insights into the use of pharmaceuticals in the mitigation of radiation-induced cancer.

The implications of our findings for 1) Future Research, 2) Risk reduction for both space exploration as well as for the general population:

The implications are clear. Our results, though preliminary, provide a glimpse of the enormous potential payoff that chemoprevention research could provide not only for the future health of astronauts exposed to radiation, but also in the general population in the battle against cancer. Regardless of the reason for an individual to be at increased risk for developing particular cancers, be it radiation exposure as in our studies (relevant to astronauts and space travel) or genetic and environmental factors (relevant to the general population), specific chemopreventive compounds and strategies can be identified and implemented to mitigate risks that predispose

individuals to cancer. Much work remains to be done to fully realize the benefits of chemoprevention strategies specifically in the battle against radiation-induced cancer. Support for research into chemoprevention of radiation-induced neoplasms such as that provided by NSBRI therefore benefits not only space exploration efforts, but what is learned in this important area also could provide unique insight into cancer chemoprevention for the general population.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Ann R. Kennedy, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures for Space Radiation Biological Effects

Project Executive Summary

Original aim

The original aim of this study was to select a formula of dietary supplements that protect against space radiation-induced biological effects, with particular emphasis on radiation-induced oxidative stress and cancer. In the initial phase of the study, which was expected to take 18 months, we have performed studies to select dietary supplement agents that are most effective in suppressing radiation-induced oxidative stress *in vitro* and in animals. It was expected that a radiation carcinogenesis study would take place after the initial phase of the study to determine the effects of the selected dietary supplement agents in preventing radiation-induced cancer development. The effects of the selected dietary supplement agents on radiation-induced oxidative stress and radiation induced carcinogenesis would then be compared to determine whether the two effects are related. The two specific aims of the first 18 months of the study were to select dietary supplement agents and agent combinations that reduce radiation induced oxidative stress in cultured cells and determine the effect of selected dietary supplement agents on radiation induced oxidative stress in Sprague-Dawley rats.

Key findings

The key findings of our research project during the first year are as follows:

- 1) We have developed and optimized a dichlorofluorescein (DCF) fluorometric assay that can reliably detect oxidative stress induced by radiation in doses as low as 1.4 cGy, and can be used to evaluate the effects of various agents on radiation induced oxidative stress *in vitro*.
- 2) We have compared the efficiency of four types of radiation in inducing oxidative stress in cultured cells and observed that γ -rays, X-rays, protons and HZE particles (1-GeV iron ions) are about equally efficient in inducing oxidative stress in cultured cells.
- 3) We have selected several candidate agents that are highly effective in preventing radiation induced oxidative stress *in vitro*. The short list of the selected agents includes ascorbic acid, N-acetyl cysteine, co-enzyme Q10, α -lipoic acid, L-selenomethionine and vitamin E succinate. These agents, when used alone or in combination, are highly effective in preventing radiation induced oxidative stress in cultured cells, and their effects are consistent and reproducible for all of the different types of radiation sources used to induce oxidative stress in these studies.
- 4) We have verified that an *in vitro* transformation system based on HTori-3 cells can be used to evaluate the effects of selected agents on malignant transformation induced by the various types of radiation.
- 5) We have observed that dietary supplementation with agents that prevent radiation induced oxidative stress enhanced the bio-reduction capacity in Sprague-Dawley rats irradiated with γ -rays or protons and prevented the reduction of bio-reduction capacity in Sprague-Dawley rats exposed to radiation with 1-GeV iron ions.

Impact of findings

The results of our experiments performed during the first year of this research project have laid a solid foundation for the research investigations described in our grant. With the development and optimization of the DCF fluorometric assay, we now have a very reliable method to detect oxidative stress induced by radiation at very low radiation doses and to evaluate the effects of candidate agents on radiation induced oxidative stress. The discoveries that the four types of radiation were about equally effective in inducing oxidative stress in cultured cells, and that the effect of the antioxidants and dietary supplement agents on radiation induced oxidative stress did not change substantially in experiments using different types of radiation, suggest that the results obtained with one type of radiation are highly indicative of the results with other types of radiation. Thus, most of the experiments needed for the development of countermeasures for space radiation induced oxidative damage in future studies can be carried out using γ -rays and/or X-rays, which are readily available for routine use in experiments. The scarce resources of protons and HZE particle radiation can be saved for the confirmation of results and validation of important findings observed in studies performed using the readily available types of radiation at the University of Pennsylvania.

We have also confirmed that the HTori-3 cell based *in vitro* transformation system can be used to evaluate the effects of candidate agents on malignant transformation induced by various types of radiation. This assay system will be used in our studies in the next year to evaluate the effects of the selected candidate agents on malignant transformation induced by HZE particle radiation.

In animal studies, we have demonstrated that the bio-reduction capacity, measured as the serum concentration of total antioxidant power, was affected by radiation exposure and can be modified by treatment with agents that prevent radiation induced oxidative stress *in vitro*. This system provides us with a means to relate our findings in the *in vitro* experiments to the results of our animal radiation experiments regarding radiation induced oxidative stress *in vivo* as well as to the results of other investigators regarding radiation induced carcinogenesis.

With the progress described here, we anticipate that we will achieve the objectives specified in our grant proposal as planned.

Proposed research plan for the coming year

Our research plan for the remaining time of the total grant period is as follows:

- 1) Repeat some of the DCF fluorometric assay experiments to determine the effects of several lipid-soluble agents on radiation induced oxidative stress. In our studies performed during the first year, we have used DMSO and ethanol as solvents for the lipid-soluble agents in most experiments. The results demonstrated that DMSO and ethanol were both highly effective as scavengers in the DCF fluorometric assay system and it is often difficult to differentiate the effects of the agents being evaluated from the effects of solvents on radiation induced oxidative stress. Recently, we have evaluated several organic solvents and found that acetone and THF are good solvents for the lipid-soluble agents being studied in our project and that these agents do not interfere with the DCF fluorometric assay in radiation experiments utilizing γ -rays. In the remaining time of this grant period, we plan to repeat the DCF fluorometric assay experiments for co-enzyme Q10, α -lipoic acid and vitamin E succinate using proton and HZE particle radiation so that the effects of these agents on oxidative stress induced by these types of radiation can be determined more precisely.

2) Perform HTori-3 cell transformation studies using HZE particles and protons as the radiation sources. We have observed that exposure to protons and 1-GeV iron ions increased the transformation of HTori-3 cells, measured as the frequency of colonies capable of anchorage-independent growth. Due to the limited beam time of the HZE particle radiation available to us (4 hours total) during the last year, we were unable to study the effects of selected candidate agents on HZE particle radiation induced malignant transformation *in vitro*. In the next year, we plan to carry out HTori-3 transformation experiments to determine the effects of selected candidate agents on HZE particle induced transformation *in vitro*. The HTori-3 transformation experiments using proton radiation will also be repeated to assure that the experimental findings observed during our first year of the study are reproducible.

3) Animal studies using proton and HZE particle radiation. Animal experiments similar to those performed last year are planned to determine the effects of selected agents and agent combinations on the bio-reduction capacity in Sprague-Dawley rats irradiated with protons and HZE particles. In the animal studies performed last year, we used lipoic acid that was not in a reduced form. We have recently determined in the DCF fluorometric assay that it is the lipoic acid in reduced form (α -lipoic acid) that is highly effective in preventing radiation induced oxidative stress. In the animal experiments to be performed in the coming year, α -lipoic acid will be incorporated into the diet for animal treatment. In addition to measuring the serum level of total antioxidant power, we plan to measure the levels of thiols and antioxidant enzymes in tissue samples to determine the effects of dietary supplementation with the selected agents or candidate agent combination on the host antioxidant defense mechanisms in animals exposed to protons and HZE particle radiation. In the original grant proposal, we planned to measure the protein carbonyl content in tissue samples as a means of quantifying radiation induced oxidative stress in tissue samples. We are still in the process of adapting the protein carbonyl content assay for measurement of oxidative stress in tissues of irradiated animals. If successful, we will measure the protein carbonyl content in the animal tissue samples collected in experiments performed last year and in the coming year.

The *in vitro* transformation studies and the studies on the host antioxidant enzymes activities mentioned above are not contained in the grant proposal. However, given the progress that we have made so far in achieving the original objectives stated in the grant proposal, we feel that we are in good position to propose these additional studies to lay the foundation for the next grant period.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Marcelo E. Vazquez, M. D., Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	CNS Damage and Countermeasures

Project Executive Summary

Space travel beyond the Earth's protective magnetic field (for example, to Mars) will involve exposure of astronauts to irradiation by high-energy nuclei such as ^{56}Fe (HZE radiation), which are a component of galactic cosmic rays. These particles have high linear energy transfer (LET) and are expected to irreversibly damage cells they traverse. Exposure to HZE radiation may therefore cause progressive deterioration of brain function, adding to other inescapable damage involved in normal aging. We propose a study of the hypothesis that long-term behavioral alterations are induced after exposure of the brain to 1 GeV/n iron particles with fluences of one to eight particles/cell targets. Previous studies support this notion but are not definitive, especially with regard to long-term effects. Our principal goal is to examine the neurological effects of high-LET radiation on C57BL/6 mice using a series of behavioral tests to unveil the temporal expression of altered behaviors in the radiation response, as well as the means, which can modulate these responses. The studies proposed in this application are designed to: 1) Characterize the behavioral consequences after exposure to low-fluences of heavy ions and protons on C57BL/6 mice. The main behavioral endpoints to be used in these studies are locomotor activity to evaluate the integrity of striatal dopaminergic pathways, and spatial reference memory to probe hippocampal cholinergic pathways. 2) Characterize the neurochemical and structural changes induced by heavy ions and protons. 3) To develop countermeasures to protect neural cell populations exposed to low fluences of heavy ions and protons. The project will test methods to protect injured neural cells based on their molecular and cellular mechanisms that may regulate neural cell survival in the central nervous system. Among the methods that will be studied is the direct administration of neuroprotective molecules as well as the modulation of apoptotic pathways by pharmacological manipulation. The effects of three different neuro/radioprotectors (GM1, melatonin and PTF- ∞) on the levels of radiation induced neurochemical and structural damage will be compared with the level of behavioral alterations to determine a cause/effect relationship.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Marcelo E. Vazquez, M. D., Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	Risk Assessment and Chemoprevention of HZE Induced CNS Damage

Project Executive Summary

Because successful operations in space depend on the performance capabilities of astronauts, radiation-induced neurological damage, could jeopardize the successful completion of mission requirements, as well as have long-term consequences on the health of astronauts. It is therefore necessary to understand the nature of this risk in order to assess its seriousness and to develop countermeasures. Compared to the large literature associated with radiation therapy, knowledge is limited about the cellular and molecular responses of cells to high-LET HZE radiation in general, and very limited about the central nervous system (CNS) specifically. Therefore, we propose to compare the effects of charged particle (Fe, Si), protons, gamma and x-ray radiation on the cells of the CNS, namely neurons and glial (astrocytes and oligodendrocytes). Cell cultures of CNS cells, both cycling and post-mitotic differentiated cells, will be utilized as model systems. We will test the hypothesis that exposure to low fluences/doses of heavy ions and protons can induce cell death in neural CNS neural cells and that increasingly dense ionizing radiation will be increasingly toxic. The activation of two separate stress signal transduction pathways will be examined (p53 and ceramide) for their role in causing cell death or other deleterious changes caused by irradiation. And with respect to p53, we will determine which of the post-translational modifications in regulating p53 function are relevant for charged particle induced cell death. Finally, we will test the hypothesis that modulating the stress signal transduction pathways will modify the radiation response of brain cells exposed to heavy ions and protons, and test the efficacy several compounds as potential countermeasures for HZE radiation toxicity.

**NSBRI RESEARCH PROGRAM
SMART MEDICAL SYSTEMS**

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RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Lawrence A. Crum, Ph.D.
ORGANIZATION:	University of Washington
PROJECT TITLE:	Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care

Project Executive Summary

Specific Aims

The principal objective of this NSBRI Smart Medical Systems Team project is to develop an image-guided ultrasound therapy system for mission critical care. In long-term space flight missions, a number of medical situations could develop that if not adequately addressed would result in mission failure. For example, although gravity is significantly reduced in space, inertia is not, and the collision of an astronaut with a heavy object could result in blunt internal trauma and it's often associated internal bleeding. In addition, as recent experiences in Antarctica demonstrate, medical conditions that require some form of surgery may well appear without warning, even when extensive pre-screening is undertaken. We are developing a smart medical device that will provide a versatile capability to treat a variety of these mission-critical medical conditions. We have demonstrated that a device that produces High Intensity Focused Ultrasound (HIFU) can be combined with a device that provides ultrasound imaging to produce a duplex system that can both image a particular condition of interest and provide therapy to relieve the condition. "Image-Guided Therapy" provides enormous potential for the treatment of a variety of medical conditions. In addition, we have demonstrated that the components of such a smart medical system can reasonably be expected to be lightweight and portable.

The Specific Aims of this proposed effort are as follows.

Specific Aim 1. To develop a combined ultrasound guidance and therapy system. This first-generation system should have the following components: (A) Laptop computer control, (B) Software control, (C) Compatibility with commercial ultrasound imaging systems, (D) Single element resonant transducer, and (E) Dynamic depth focusing.

Specific Aim 2. To perform studies on the combined system that would lead to optimal performance parameters. Among the studies to be performed are the following: (A) Biological effects, tissue necrosis, acoustic hemostasis, (B) Image quality for diagnosis, (C) Targeting and monitoring capabilities, (D) Acoustic focusing and power requirements, and (E) Thermal focusing limitations.

Specific Aim 3. Utilizing the results of SAs 1 & 2, to develop an integrated ultrasound guidance and therapy system. This second-generation system would have the following characteristics: (A) Integrated imaging and HIFU therapy transducers, (B) Cavitation feedback, (C) Attenuation/thermal feedback to localize treatment site, (D) Perfusion and back-scatter imaging for treatment localization, and (E) Software control and user-friendly interface.

Key Findings

Three key findings are most important to our critical path. One, as reported last year, Aim 1 has been achieved and a small portable image-guided HIFU system has been constructed. In year 2, the system was improved. Most importantly a new power supply that decreased the system

weight by over 50 percent was developed and rigorously tested. Through an NIH SBIR grant, and with our participation, a commercial partner is now marketing compact power supplies for HIFU. Two, significant strides were made toward optimizing performance (Aim 2). Most importantly, we showed that doubling the acoustic amplitude of the source cuts the treatment time by more than half. In fact in some experiments, the energy required to denature proteins was reduced by a factor of three when the pressure was increased by a factor of 1.4 ($\sqrt{2}$). Three, a provisional patent application was submitted for a circuit to permit real-time synchronization of the HIFU therapy with any ultrasound imager.

Three other key findings are also important to our SMS. One, Dr. Shahram Vaezy led the discovery that HIFU produced both hemostasis and pneumostasis in the punctured lung (Aim 2). Although we had shown that we could use HIFU to induce hemostasis in the liver, spleen, and peripheral vessels, air in the lungs which reflects ultrasound made trauma treatment in the lung an unknown. It turns out the lung is sealed more quickly than liver or spleen. Two, Dr. Peter Kaczkowski led the development of a new algorithm to monitor HIFU therapy using RF data acquired from an ultrasound imager (Aim 3). Three, in collaboration with Prof. Y. Matsumoto at the University of Tokyo, we discovered that we could use HIFU to break kidney stones.

Impact

The device's components now weigh less than 10 kg, down from more than 30 kg a year ago, and 40 kg at the start of the project. They can be packaged into a single metal chassis (the laptop computer is no longer required) and operate with the Philips HDI-5000 ultrasound imager on the ISS or whatever imager is chosen for long-duration missions (i.e., a Terason or a Sonosite imager such as used in the SMS project lead by Dr. James Thomas). The new circuit makes it possible to apply HIFU therapy while imaging in a Pulsed Wave Doppler mode.

We have established a protocol to deliver short-duration, high-amplitude pulses produced by the dynamic focusing transducer over a larger spatial area by moving the focus in time; this protocol enables us to treat large areas rapidly. This includes sealing exposed lung from blood and air leakage. These findings expand our trauma care capabilities, improve therapy efficiency, and minimize the power draw required by the device.

We have also developed a fully integrated laboratory system to detect cavitation by capturing the raw RF data and using a new algorithm to monitor lesion formation by observing changes in tissue attenuation. The system is more sensitive than standard B-mode imaging and can be used to assess the completeness of a volume treatment after cavitation bubbles have been reabsorbed by the body.

Lastly, Renal Stones Formation is Risk 12 on the Bioastronautics Critical Path Roadmap and the device we are currently building is being evaluated as a potential method to comminute renal calculi that do form in astronauts during space flight.

Research Plan for Coming Year

The current system is to be packaged in one chassis and tested on animals. The circuit for synchronization will be converted from breadboard to a printed circuit board. Using the technology developed for synchronizing with an imager we plan to develop a Pulsed wave Doppler ultrasound imaging capability with the therapy transducer. Therefore, only one transducer (no imager) is needed to target a bleed. This capability will be integrated into the system. In a continuing effort to optimize therapy we have developed a dual frequency transducer which mixes a low and a high frequency wave. Preliminary evidence shows that the

low frequency transducer increases cavitation and expands the treated region. We plan a careful study to determine if mixing frequencies can accelerate hemostasis on large bleeds by expanding the focal region. Supporting grants have been submitted to investigate targeting and treating kidney stones with ultrasound and to commercialize our prototype HIFU therapy system.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Peter F. Davies, Ph.D.
ORGANIZATION:	University of Pennsylvania
PROJECT TITLE:	Vascular Genomics in Gravitational Transitions

Project Executive Summary

Aims

When changes in the biomechanical environment of the circulation occur, blood vessels undergo well-orchestrated structural and metabolic remodeling to restore optimal function. We propose that this remarkable adaptive ability lies at the center of orthostatic intolerance exhibited by most astronauts on return to earth's gravitational field after modest-to-long periods in microgravity. We are therefore mapping gene expression (transcription profiling) of the different vascular steady states exhibited *in vivo* (mouse) in simulated hypergravity and microgravity, and the transitions between them, in order to design better countermeasures for undesired vascular consequences in long-term space flight. In particular, the transition to hypergravity will simulate the effects experienced by astronauts upon return to a significant gravitational field (Earth, Mars) following adaptation to long periods of microgravity. The studies will generate a reference genomics database identifying gene expression changes in the arteries, heart and lungs induced by gravitational shifts and mining of such databases will provide a guide to potential countermeasures to offset deleterious effects.

Key Findings

During the first year we refined the antisense RNA techniques necessary to amplify RNA from small numbers of cells with high fidelity. This became necessary when it was apparent that no literature existed for a rigorous test of the protocols required in the mouse experiments. In a model experiment, vascular cells were stimulated with the cytokine TNF for which a small number of genes are known (through conventional Northern analyses) to change. RNA from the same pool was analyzed by microarray with and without amplification. Sophisticated bioinformatics analysis of 13,800 genes was performed. The data from unamplified and amplified RNA were analyzed for fidelity, sensitivity and utility. The expected prominent changes in known genes were detected in both groups with high retention of accuracy, an essential requirement for the proposed *in vivo* gravity experiments. An interesting additional and unexpected finding is that RNA amplification increased the detection rate of genes whose differential expression was just below a significance threshold in the unamplified assay i.e. greater sensitivity of detection of differential gene expression conferred by the linear amplification techniques employed. Most important, these differences were confirmed by real-time quantitative PCR of unamplified RNA. This work was published in the journal *Physiological Genomics* in April 2003. The studies were a prerequisite for the gravitational experiments because no such analysis existed that rigorously evaluated the accuracy of the transcription profiles arising from amplification of small amounts of blood vessel.

In extending the RNA amplification techniques we next addressed differential vascular cell gene expression in two sites in the aorta of the normal adult pig. Endothelial cell mRNA was isolated from two regions of the aortic arch characteristic of disturbed flow (pro-atherogenic) and undisturbed flow respectively. RNA from paired sites in individual aortas (n=8) was isolated, linearly amplified, reverse transcribed, and cDNA was hybridized to microarrays custom-prepared from the University of Toronto human cDNA cardiovascular database (~8000 genes)

plus several thousand proprietary Incyte clones. Bioinformatics analyses identified expression patterns in the disturbed flow region indicative of an antioxidant endothelial profile that may be protective of a pro-inflammatory state. Some genes associated with major mechanisms believed to initiate atherogenesis, eg pro-inflammation, were elevated but the critical adhesion molecules necessary to initiate inflammation were not differentially expressed in this region, consistent with the absence of any pathology by histological assessment. *This is an intriguing result that demonstrates the power of this approach in identifying the interactions of multiple genes need to be considered in defining atheroprotective or susceptible situations.* As far as we are aware, these are the first high throughput array analyses of arterial endothelial gene expression directly obtained from discrete regions of blood vessel. When compared with several studies that have profiled the effects of different flow treatments on cultured (as opposed to *in vivo*) cells, many differences of gene pathways were noted. This work has been submitted to the journal *Proceedings National Academy of Sciences USA*. While these studies were performed with larger blood vessels (porcine) in order to obtain enough lining cells (endothelium), the cell numbers used are comparable to, in fact less than, those we will obtain from whole mouse aorta for the gravity studies. Techniques for the dissection of mouse blood vessels, RNA isolation and amplification has been verified under normal gravitational conditions. These evaluative experiments demonstrate that we can successfully perform the entire sets of protocols from tissue isolation to bioinformatics and gene annotation prior to the gravitational shift experiments at NASA-Ames Research Center.

Hypergravity and transitional procedures on 96 mice at NASA Ames, using the 24-foot centrifuge in close collaboration with Ames staff members, is under way. Arterial, heart, and lung tissues harvested will then be analyzed at Penn. The molecular biology is demanding and lengthy, and the bioinformatics is complex. As in the case of our publications to date, we are taking steps to ensure that the data are openly available to the widest scientific community.

Impact

New techniques addressing vascular genomics have been developed, tested, and have withstood critical peer review in leading journals in the field of genomics and biology. We are now implementing them in carefully designed experiments in which gravitational shift is the variable.

Plans for the Coming Year

For studies of hypergravitational changes, the facilities of the Chronic Hypergravity Exposure Centrifuge at NASA Ames are suitable for long-term exposure of mice at 3G to simulate return to earth (or landing on Mars surface) after long term space travel. The current experiments will provide the conditions for database development.

Mice are exposed to micro or hyper gravity for up to 28 days and the effects upon gene expression in the major arterial system, heart and lungs will be measured by the techniques outlined above. Reversal of the adapted condition will also be evaluated on a temporal basis.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Scott A. Dulchavsky, M.D., Ph.D.
ORGANIZATION:	Henry Ford Health System
PROJECT TITLE:	Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies

Project Executive Summary

Surgical disease and trauma are rated at the highest level by NASA experts in terms of probable incidence versus impact on mission and health. Abdominal pathology, dental and sinus infections, musculo-skeletal injury, ocular trauma, and urologic disorders may cause serious mission ending health consequences during space exploration. The diagnosis and management of acute health problems in space is problematic due to limited training of the Crew Medical Officer (CMO), human and environmental factors, and a lack of reference of the changes in anatomy, disease presentation, and therapy in microgravity. There is no planned radiological capability aboard the ISS, further complicating medical diagnosis in space.

Recent terrestrial investigations suggest expanded clinical applications of ultrasound and laparoscopy with miniature instrumentation. This proposal will initially determine the diagnostic utility of ultrasound and/or micro-laparoscopy in select health contingencies with high potential mission impact. These diagnostic modalities will then be used to facilitate minimally invasive, definitive surgical therapy of selected contingencies in animal models in ground based and simulated microgravity scenarios. Optimal just-in-time training regimens and refresher modules for non-physician CMOs to accomplish these tasks will be developed to answer the specific aims:

- What is the sensitivity and specificity of ultrasound or micro-invasive laparoscopy performed by experts versus just-in-time trained CMOs in the diagnosis of serious health contingencies which may occur during space exploration?
- What are the alterations in disease prevention, diagnosis, and human factor requirements for use of ultrasound/mini-laparoscopy in microgravity?
- What are the training and support requirements for physician and non-physician CMOs for optimal on-site and telemedicine diagnosis of these clinical conditions by ultrasound or video mini-laparoscopy?

The unique constraints imposed by the space environment require the development of novel diagnostic and therapeutic strategies for crew member health problems including the expansion of ultrasound and mini-laparoscopy. Thoracic ultrasound, initially investigated by NASA as an alternative diagnostic modality for pneumothorax, has proven accuracy in terrestrial and microgravity applications and has widespread impact in acute care on Earth in the future. Although some of the techniques investigated in this proposal are appropriate only for a microgravity environment, the majority of the diagnostic and therapeutic algorithms are readily transferable to terrestrial medicine including rural and military applications. The expanded use of the diagnostic and training modalities described in this proposal, if verified, would provide a significant, clinically relevant advance in space medicine capabilities with profound Earth-based ramifications.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Mark S. Klempner, M.D.
ORGANIZATION:	Boston University
PROJECT TITLE:	Smart Medical System for Detection of Microorganisms

Project Executive Summary

The goal of this program is to develop a revolutionary, non-culture based microbial detection, identification and quantification system that can be used as part of a Smart Medical System for exploratory space travel. Rapid detection and identification of microorganisms are critical to many military and civilian applications ranging from food and water safety monitoring, biological warfare agent detection and to diagnostic microbiology of human and other biological specimens. For long-term exploratory space travel there will be a critical need for a smart medical system to monitor the air and water supply for microbial contaminants, as well as an intermittent need for assessment of biological specimens from symptomatic astronauts.

Current microbial identification systems are based on the gold standard of *in vitro* culture or DNA/RNA fingerprinting. Both require considerable sample manipulation, delay in readout, are semiquantitative and subject to interfering substances and contamination, and require additional processing to resolve complex mixtures of microorganisms. This proposal involves the development of a novel smart medical system to detect and identify bacteria through the use of microsensors and includes three steps: 1) Development of “fingerprinting” phage display libraries which can detect, identify, quantify and discriminate bacterial species in environmental and biological specimens; 2) Application of phage displayed peptides and antibody fragments in a microarray to the surface of a microsensor to demonstrate the microarray microbial fingerprint response to selected bacterial species using optical readout and electronic MEMS resonator arrays and to characterize the sensitivity and specificity for detecting and discriminating between bacterial species using surface “fingerprints;” and 3) Development of algorithms from the microarray response for the real time identification and discrimination of bacterial species.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Lakshmi Putcha, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration

Project Executive Summary

A continuing challenge for space medical operations at NASA is the management of pathology associated with neurovestibular adaptation during space flight. A primary manifestation of this problem, particularly in the first few flight days of shuttle missions, is space motion sickness (SMS). The current treatment of choice for symptoms associated with SMS is promethazine (PMZ). Although oral tablets and rectal suppositories have been used during space flights, the intramuscular route appears to be most effective. On the other hand, intramuscular administration of drugs is an invasive procedure and PMZ causes irritation at the site of injection. A key research topic in the Smart Medical Systems area of the NSBRI 99-02 research announcement is development of novel therapeutic modalities for remote site medical operations such as space missions. In response to this initiative, the goal of the proposed research is to develop an intranasal dosage formulation of PMZ that will provide crewmembers with a non-invasive means of self-administering SMS medications. Accordingly, the following three aims will be addressed: 1) Develop a microencapsulated, pH-balanced gel dosage formulation and a combination form with a corticosteroid for intranasal administration of PMZ; 2) Establish the release kinetics and shelf life of the optimized dosage forms; and 3) Assess bioavailability, nasal mucosal irritability and toxicity of the selected dosage forms in rats.

The proposed formulation development will focus on tailoring the release characteristics of the dosage form to optimize therapeutic index and minimize irritability at the site of administration. Once the optimal dosage form has been identified based on release kinetics and stability characteristics, bioavailability, nasal irritability and toxicity after single and multiple dose administration will be assessed in an animal model. Development of an intranasal drug delivery system for motion sickness treatment will benefit pharmacotherapeutics in space as well as on Earth.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
ORGANIZATION:	University of Massachusetts Medical School
PROJECT TITLE:	Noninvasive Measurement of Blood and Tissue Chemistry

Project Executive Summary

The hypothesis for this project is that near infrared spectroscopy (NIRS), in combination with unique statistical methods, can be used to noninvasively measure blood and tissue chemistry for any human subject. We plan to develop new NIRS methods which will enhance this promising technology so it can be used on all humans, irrespective of skin color and gender. We plan to demonstrate this new approach by developing techniques to noninvasively measure blood hematocrit and tissue pH and PO₂ (partial pressure of oxygen) on human surgical and ICU patients. We will then demonstrate how the combination of these parameters can be used to diagnose shock and hypovolemia and guide resuscitative therapies. This system will serve as a platform for NIR measurement of multiple parameters and the development of computerized algorithms to assist in the diagnosis and treatment of both acute and chronic medical conditions. The specific system demonstrated in this proposal is intended to evolve into a medical monitoring system for use during extended spaceflight, but will also find immediate application in terrestrial hospitals, emergency vehicles and emergency rooms.

The Specific Aims as originally described in the research proposal are:

- 1: Develop methodology to statistically describe NIRS spectral variations due to ethnicity, fat content and light scattering.
- 2: Develop and validate noninvasive methods for determining tissue pH and oxygenation from the NIRS spectra of human subjects.
- 3: Establish algorithms for the use of tissue pH, oxygenation and hematocrit measurements to indicate shock and hypovolemia and to guide resuscitative treatment.
- 4: Specify requirements for miniature, lightweight optical device.

Year 2 Progress Toward These Aims

In our year 1 report we demonstrated methodology for deriving factors to correct for skin color. We also derived corrections for fat content. We evaluated these factors for their ability to improve the accuracy of blood hematocrit (Hct) measurement on multi-ethnic subjects. The goal is be able to noninvasively measure blood hematocrit with an accuracy of <2 Hct percent and an $R^2 > 0.75$ on an independent group of subjects. In our original proposal we hypothesized that removal of skin color and fat content should be sufficient to reach our goals. During year 1, we found that these factors alone were not sufficient to meet the accuracy goals. In year 2 we evaluated additional factors, as well as new methods for developing multivariate calibration equations.

During Year 2 we have shown that subject variability in skin color, sensor placement, light scattering and instrumentation all limit accuracy of the NIRS measurement. Early efforts to model these variations showed modest results. More work is necessary to optimize the derivation of the correction factors and improve the instrumentation. Only 20 – 30 subjects should be

needed to describe spectral variability in each of the 3 analytes (Hct, tissue pH, tissue PO₂). If subject variability can be modeled with a separate set of data, it can be applied to the measurement of all three analytes and any new analytes we plan to measure in the future.

In Year 1 we demonstrated excellent accuracy for single subject calibration models for tissue pH and PO₂ on cardiac surgery patients. Models were developed from a portion of the data collected from each patient and tested on data from that patient not used in the model. The development of models that were predictive of completely independent subjects was limited by problems with the light source used to collect the data. The light source was repaired and we planned to develop these models from subjects in the ICU. During Year 2 we planned to recruit ICU patients at both UMass Medical School and the University of Pittsburgh Hospital.

During Year 2 the recruitment of ICU subjects at risk for sepsis and multiple organ failure was more difficult than anticipated, primarily because patient's feared infection from the invasive sensor. When subjects were recruited, it was difficult to keep the invasive sensor in place, because the patients moved their hands and the patients themselves were transported; these events dislodged the invasive sensor. In one patient, monitored for 3 days did observe the signature expected for sepsis: depressed pH with constant PO₂. We have suspended data collection from ICU patients until we have made further developments in sensor technology, which we anticipate will improve our ability to successfully monitor these patients.

Aims 1 and 2 have provided us with sufficient data to determine the requirements for a miniature, lightweight optical system. Advancements in miniature spectrometer technology have allowed us to consider the use of a full spectrum lightsource for the miniature instrument. The requirement for multiple correction factors make the use of a spectrometer, rather than LED's a less risky choice for the next generation system.

We have been awarded a contract by the US Army Medical Research Command to design a miniature system to simultaneously measure tissue pH, PO₂ and blood hematocrit. Based on the Aim 1 and 2 results from the NSBRI project, the primary objective for the new hardware is to reduce system and placement variations between subjects. Combined with our advancements in software correction factors we anticipate meeting the accuracy targets required for NIRS measurements.

Future Research Plans and Implications for Project Findings

In Years 1 and 2 we demonstrated that tissue pH and PO₂ can be measured noninvasively using near infrared spectroscopy and has the ability to detect very small changes in muscle blood flow and metabolic demand. During Year 3 of this project we will see a shift in direction to address NASA's request to focus our technology on a exercise, rather than trauma application. The ultimate use of the deep muscle pH and PO₂ sensor would be for fitness assessment and development of countermeasures to preserve muscle mass, strength and endurance. In response to this request, we will modify the design of our fiber optic sensor to enable us to accurately probe muscle pH and PO₂ deep within the quadriceps muscle. We plan to begin validation of this sensor against an invasive "gold standard". This project would continue into Year 4 and 5 to perform validation studies of the noninvasive sensor at JSC's exercise physiology lab using their standard protocols.

An accurate muscle sensor will facilitate collection of data from ICU patients, enabling us to return to building a database for the identification and development of treatment algorithms based on tissue pH, PO₂ and hematocrit. We envision that these treatment algorithms will be part of a smart medical system used to respond to trauma. Such a system will find use for long

duration space flight, but will also be valuable on earth, first in the hands of the Army's medics, and eventually for first responders to medical emergencies on our highways and in our cities.

We have learned that instrument stability and repeatable sensor placement are more important than skin color and fat content variation for achieving accurate measurement for all subjects. Simultaneous with the development of the deep muscle sensor we will continue our algorithm and hardware development to model and control subject-to-subject variability that degrades accuracy of all NIRS measurement techniques. The hardware development will take place as part of a complementary project sponsored by the US Army Medical Research Command. We will attempt to incorporate these improvements as they become available. The advances made in hardware and algorithm design will be applicable to any NIRS transdermal measurement and will help advance the development of a single device which will noninvasively measure multiple clinical parameters.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Jeffrey P. Sutton, M.D. Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Near Infrared Brain Imaging for Space Medicine

Project Executive Summary

This project is part of the NSBRI Smart Medical Systems Team. It develops and applies a new technology, diffuse optical tomography (DOT), for portable non-invasive functional monitoring suitable for neuroimaging in space. The technology has potential capabilities to:

- Quantitatively assess physiological adaptation (e.g., changes in intracranial pressure and blood flow) associated with microgravity;
- Detect regional brain activity correlated with performance under altered circadian and mental loads;
- Provide remote clinical assessment; and
- Guide treatment.

As assessed by NASA, the project addresses two of the four highest priority risk areas on the Critical Path Roadmap (CPR). These are (1) trauma and acute medical problems, and (2) human performance failure because of poor psychosocial adaptation. In addition to applications for space, the developments of this project have relevance to Earth medical research and care. For example, DOT is now being tested for monitoring stroke progression in patients with acute cerebrovascular accidents.

The project brings together scientists, engineers and physicians at Baylor College of Medicine, Massachusetts General Hospital/Harvard-MIT Division Health Sciences and Technology, and medical operations personnel at NASA Johnson Space Center (JSC), to work collaboratively on the development and testing of DOT as a space relevant technology. The **original aims** are to:

1. Refine current DOT technology to build an instrument with improved spatial and temporal resolution to detect brain activity non-invasively, and in real time, through the intact skull;
2. Validate the improved instrument using functional magnetic resonance imaging (fMRI), which is a standard technology, and test DOT as a portable brain imaging device for assessing motor and cognitive activity under normal and sleep deprived conditions in normal human subjects;
3. Assess DOT, along with optical coherence tomography (OCT), to non-invasively measure altered intracranial pressure (ICP) in neurological patients, given that altered ICP may occur in the space environment; and
4. Refine a system for automated image interpretation using individualized brain models and computational techniques.

During the second year of this award, **key findings** and accomplishments include:

- Further refinement of DOT instrumentation to validate the technology using fMRI;
- Demonstration and publication of the excellent temporal correlation between DOT and fMRI brain activity across time during simple motor tasks;
- Simultaneous optical/fMRI brain imaging of subjects as they perform our space relevant visuomotor performance task, SpaceDock, and re-imaging of these subjects following ~30 hours of prior sleep deprivation (to simulate the sleep deprivation stress of spaceflight);
- Observation of significantly altered brain activity following sleep deprivation, including detection of brain *deactivation* in the frontal pole associated with task performance (found with both fMRI and DOT); and
- Development of a demonstration system for automated multi-sensor analysis and interpretation (Automated Intelligent Medical System; AIMS), which can use sensor information from multiple astronauts and/or the environment to make medical diagnoses.

The **impact of these findings** are that they address the technology requirements set forth in Aim One, and provide validation for DOT as laid out in the objectives and hypotheses of Aim Two. The motor data using simultaneous DOT and fMRI confirm one of the hypotheses contained in Aim Two, namely that DOT and fMRI will be able to detect changes in regional brain activity contralateral to motor movement. The DOT/fMRI findings using the SpaceDock task confirm the hypothesis that the DOT technique can detect frontal cortex changes as a function of mental load in normal and sleep-deprived subjects. The AIMS system provides a general-purpose platform for simultaneously and autonomously monitoring many sensors, and speaks to the issue of individualized, digitized human, anatomical brain models upon which time-derivative functional data are used for interpretation and display in real time (Aim Four).

In the coming year, the **proposed research plan** follows the timeline described in the initial proposal. The project is on schedule for all milestones. In year three, further DOT instrument and probe design and development will be achieved. Publication of the SpaceDock platform design, as will the results obtained from normal and sleep-deprivation conditions. Patient populations with elevated ICP will be investigated. Finally, continued development will take place on the AIMS informatics system towards more automated machine/human interface, applicable to DOT imaging as well as other, analog settings.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	James D. Thomas, M.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications

Project Executive Summary

The NSBRI has identified that the efficient and automated delivery of health care in space is a key research arena for the future. Specifically, they propose to develop a “Smart Medical System” that will be able to monitor crew health, identify deviations from ground-based norms, and allow timely intervention by crew members who may have only a moderate amount of training in medicine. For the last three years, the principal investigator and colleagues have worked closely with NASA scientists, flight surgeons, and engineers to optimize research and diagnostic ultrasound aboard the International Space Station (ISS) and thus are well positioned to develop the necessary tools and techniques to integrate ultrasound into the Smart Medical System. A principal limitation of ultrasound technology is its extreme dependence on the expertise of both the acquiring examiner and the interpreting physician. This is particularly true of two-dimension ultrasound, where the examiner is required to obtain precisely oriented anatomical sections of the organ of interest.

Three-dimensional ultrasound has the advantage of acquiring a large anatomic volume from a single ultrasonic window, and thus may be less dependent upon the expertise of the examiner. Furthermore, this large volume may contain sufficient anatomic landmarks to allow unambiguous registration with previously obtained three-dimension data from either ultrasound or other modalities such as magnetic resonance imaging (MRI) or computed tomography (CT). One could thus envision a system by which whole organs or even the entire body would be imaged in three-dimensions prior to launch; data which could be used to compare with subsequently obtained three-dimensional data sets using in-flight ultrasonography. The overall purpose of this grant is therefore to perform ground-based research, development, and validation aimed at optimizing diagnostic ultrasound in manned space flight, with the following general hypothesis:

Unifying hypothesis: Serial three-dimensional ultrasound examinations will enhance diagnostic capabilities in manned space flight.

The technical aspects of this program will be pursued with the following specific aims:

1. Optimize the acquisition methods for three-dimensional sonography, utilizing reconstruction and real-time techniques.
2. Develop techniques for registering anatomical images from two- and three-dimensional ultrasound with those obtained from prior ultrasound examination and from magnetic resonance and computed tomographic imaging, considered “gold standards” for non-invasive anatomical imaging.
3. Develop tools for abstracting, in an automated fashion, anatomical changes from serial three-dimension and two-dimension ultrasound studies.

4. Develop algorithms for the optimal compression of three-dimensional ultrasound images and refine current two-dimensional compression algorithms.
5. Assess the ability of novice examiners to obtain three-dimensional sonographic data sets following minimal training.

These objectives will be pursued using data from a variety of *in vitro*, animal and clinical models. In particular, we will take advantage of a well-established collaboration with the National Institutes of Health, which permits highly sophisticated chronic animal models to be examined with a minimum of additional resources. Although the tools developed here should be applicable to any organ of the body, we will focus our efforts on the kidneys and the heart.

At the conclusion of this project, we anticipate delivering to the NSBRI and its Smart Medical System a set of algorithms and software for the non-rigid morphological registration and comparison of serial two- and three-dimensional ultrasound data sets and validated algorithms for optimal compression of four-dimensional ultrasound data. In addition to these technical deliverables, our validation work on nephrolithiasis will provide important diagnostic clues for assessing this condition in manned space flight. Similarly, the work on cardiac mass regression following unloading will be invaluable to the NASA research and medical operations community in assessing the impact of long-term space flight on cardiac atrophy and utility of prophylactic countermeasures.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	James D. Thomas, M.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity

Project Executive Summary

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations generally are well tolerated, but upon return to Earth, astronauts often suffer from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities in the myocardium itself develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes.

Alone among such imaging modalities of radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Indeed, the Science Working Group (SWG) for the International Space Station (ISS) Human Research Facility (HRF) has recognized the primacy of ultrasound for medical diagnosis and physiology research, with plans to launch a specially modified commercial ultrasound instrument to the ISS in 2001. Echocardiography is similarly being used before and after shuttle flights and in a variety of bed-rest studies sponsored by NSBRI and NASA. Unfortunately, while ultrasound has the potential for high spatial and temporal resolution imaging of the heart, in the past it has been severely limited by operator inexperience and inconsistency in its subjective interpretation. Needed are new methodologies for assessing the load-independent function of the heart and consistent, objective quantification of a wide range of NASA echo studies, whether obtained on the ground, in flight or in experimental models. We propose to provide such a facility while validating novel methods for the load independent assessment of myocardial function. Our central hypothesis is that:

Microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties.

A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS. However, within the scope of this grant, we propose several specific aims that will be critical to the ultimate comprehensive study of the cardiovascular system in space. Key issues: 1) Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates ($e's$, $e'd$); 2) Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the

early detection of myocardial dysfunction during prolonged space flight. Additional deliverables to NSBRI: 3) Development and distribution of stand-alone software and algorithms for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources; 4) Establishment of an Echocardiographic Core Facility to the NASA research and clinical community, capable of applying standard and novel analysis techniques in a rigorous fashion to echocardiographic data obtained from selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.

If successfully implemented, these aims will allow the cardiovascular sequelae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

**NSBRI RESEARCH PROGRAM
TECHNOLOGY DEVELOPMENT**

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Associate				
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RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Isaac N. Bankman, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Development of a Space Qualifiable MRI System

Project Executive Summary

This proposal is to develop a proof-of-concept engineering model of a space qualified Magnetic Resonance Imaging (MRI) system for small animals and astronaut limbs with mass of < 130 kg and average power when on but not scanning < 1 kW and when scanning < 1.2 kW, not including the processor. An onboard processor or a high-performance PC can be adapted. MRI provides high-resolution, high-quality anatomical information without ionizing radiation so it can be safely used repeatedly to track changes without deleterious effects. As a result, the study of physiological alterations in space and the development, verification, and maintenance of countermeasures will be significantly enhanced. Mice and small rat models are useful surrogates to carry out in-orbit physiological studies. Measuring alterations in the limbs of astronauts, especially the lower limbs, will provide partial confirmation of the effectiveness of proposed countermeasures and the utility of Earth-based animal models. In-flight MR imaging of mice and rats will especially benefit the countermeasure developments of several of the NSBRI research teams. The proposed concept is based on traditional MRI principles and uses advanced technology and advanced engineering techniques to reduce mass and power to acceptable levels. The system consists of a 1 to 1.5 Tesla cryogen-free high temperature superconducting magnet subsystem and advanced electronics that will have magnetic field inhomogeneities ≤ 8 ppm over a spherical imaging volume of 10 cm diameter and ≤ 10 ppm out to 15 cm diameter. The magnet cryocooler subsystem will be designed using high temperature superconducting materials to significantly reduce the mass and power of the cryocooler. The highest resolution mode gives a resolution of 117 microns for small animals over a spherical imaging volume of 6 cm diameter and a resolution of 352 microns for human limbs over a spherical imaging volume of 18 cm diameter. The standard resolution mode will provide a resolution of 234 microns and 703 microns, respectively. The pulse sequence scenarios used will be those traditionally used in MR imaging to achieve images that are proton-density, T1 or T2 weighted so that a significant amount of structural information will be available. Because of budget limitations, only selected electronics will be reengineered to demonstrate the minimum mass and power that can be achieved. We ask that the panel consider a supplemental budget request that allows redesign and fabrication of all of the electronics to minimize mass and power. The team is composed of individuals and organizations with a unique combination of expertise including: MRI systems development at the General Electric Research and Development Center, advanced MRI development and small animal experimentation at the Johns Hopkins School of Medicine, and the development of reliable medical and low-mass, low-power systems for space applications at the Johns Hopkins University Applied Physics Laboratory.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Jay C. Buckley, M.D.
ORGANIZATION:	Dartmouth Hitchcock Medical Center
PROJECT TITLE:	Improved Bubble Detection for EVA

Project Executive Summary

The objective of this project is to improve EVA efficiency and safety through the *in-vivo* validation of a unique ultrasonic bubble-sizing and detection instrument. This instrument exploits bubble resonance by using two frequencies of ultrasound (dual-frequency ultrasound) to detect and size bubbles in tissue and blood. The original aims of the project were to: (a) establish the appropriate transducer configurations, electronic settings and instrument enhancements to detect and size bubbles reliably *in-vivo*, (b) compare the new bubble monitoring technique to Doppler, and use it to investigate decompression sickness and (c) develop the capability to size small bubbles in tissue. The progress toward each of these aims is summarized below.

Progress

Progress toward establishing the appropriate transducer configurations, electronic settings and instrument enhancements to detect and size bubbles reliably *in vivo* – This aim has been accomplished. This was done using a stepwise approach. First, experiments were performed *in-vivo* using agitated saline (the agitated saline contains small bubbles). The transducers of the dual frequency device were aimed into the right ventricle. Agitated saline was injected intravenously while data were collected with the device. These experiments demonstrated that bubbles could be detected as they move through the right ventricle and right atrium. These experiments established the technical knowledge (transducer position relative to anatomical features, equipment settings, etc.) needed to monitor bubbles during subsequent decompression experiments.

In the decompression experiments, the transducers were positioned on the chest wall and the ultrasonic energy was beamed into the right ventricle. The pump frequency (which selects the size of bubble that will be imaged) was increased stepwise from 30 kHz to 180 kHz in 5 kHz increments. At each frequency data were taken with the pump transducer on and off. By comparing the signals returned with the pump on to that with the pump off for each pump frequency, a histogram of bubble sizes could be produced. This work is significant, since the ability to produce bubble size histograms during decompression stress is a new capability that may have both operational and research uses.

Progress toward comparing the new bubble monitoring technique to Doppler, and using it to investigate decompression sickness – This aim has been advanced by comparing the signals obtained with the dual frequency device to a standard clinical ultrasound instrument. Early indications are that the dual frequency device may detect bubbles prior to Doppler, but work in this area is ongoing. A Doppler capability has also been added to the dual frequency device, primarily to assist in aiming the transducers at the ventricle but also as a complementary means to signify the presence of bubbles.

The combination of the Doppler and dual frequency ultrasound is being used to: (a) evaluate the changes in bubble size during the evolution of decompression sickness and (b) evaluate perfluorocarbons as a potential treatment for decompression sickness. The combination of the

two bubble detection capabilities into one device provides a versatile instrument for studying decompression sickness.

Progress toward developing the capability to size small bubbles in tissue – This aim has been advanced through a variety of *in vitro* and *in vivo* studies. The tissue bubble detection effort has two goals: (a) to evaluate whether very small bubbles (< 30 microns) can be detected in tissue, since decompression sickness theory suggests that small bubbles may exist in tissue normally at ambient pressure and (b) to detect larger bubbles in tissue and in the vasculature that may cause symptoms during decompression sickness. Current efforts have focused on the first goal. Early studies demonstrated signals consistent with bubbles in the thigh of the swine. These signals were found only at particular locations. In the original implementation of the detector, however, the source of the mixing signals could not be determined because of poor spatial resolution. The modified bubble detector now allows for sampling at selected depths, so the mixing signals can be correlated with anatomic structures. This has shown that mixing signals are most likely returned from interfascial planes, i.e. the areas between muscle groups.

Validating tissue bubble detection requires a reliable way to demonstrate that signals detected in tissue originate from bubbles. Current research is focused on developing *in-vitro* tissue bubble simulators capable of generating prototypical small bubbles to test the tissue bubble detection equipment. Several *in-vitro* methods are under evaluation, including contrast agent embedded in gelatin, decompressed gelatin, and schemes involving the passage of high-pressure air through very small pipettes.

The tissue bubble detection work is significant since the ability to detect and size bubbles in tissue would be a new and unique capability.

Plan for the Coming Year

In the coming year the plans are to:

- Refine the bubble size histogram capability;
- Use histograms to evaluate bubble sizes during decompression stress and during interventions to treat decompression sickness (e.g. administration of perfluorocarbons);
- Advance tissue bubble detection; and
- Pursue human use approval for the device.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Harry K. Charles, Jr., Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	AMPDXA Scanner for Precision Bone and Muscle Loss Measurements During Long-Term Space Flight

Project Executive Summary

The purpose of the Advanced Multiple Projection Dual Energy X-ray Absorptiometry (AMPDXA) Scanning System project is to design, build, and test a precision scanner system for monitoring the deleterious effects of weightlessness on the human musculoskeletal system during prolonged spaceflight. The instrument uses dual energy X-ray absorptiometry (DXA) principles and is designed to measure bone mineral density (BMD), decompose soft tissue into fat and muscle, and derive structural properties (cross-sections, moments of inertia). Such data permits assessment of microgravity effects on bone and muscle and the associated fracture risk upon returning to planetary gravity levels. Multiple projections, coupled with axial translation, provide three-dimensional geometric properties suitable for accurate structural analysis. This structural analysis, coupled with bone models and estimated loads, defines the fracture risk. The scanner will be designed to minimize volume and mass (46 kg goal), while maintaining the required mechanical stability for high-precision measurement. The AMPDXA will be able to detect one percent changes in bone mass and geometry and five percent changes in muscle mass.

The AMPDXA project is a joint effort between the NSBRI's Technology Development Team, the Bone Demineralization/Calcium Metabolism Team, and the Muscle Alterations and Atrophy Team. Its goal is to provide the high precision monitoring system necessary to fully assess both the deleterious effects of weightlessness on the bones and muscles and the effectiveness of any countermeasures. We believe that any pharmacological or exercise-related countermeasures used by astronauts to mitigate microgravity effects will require efficient and timely monitoring. Moreover, the monitoring device must be capable of being used by astronauts during spaceflight so that feedback can be dynamically employed to regulate countermeasure doses. The system design will be such that intelligent, but not necessarily medically trained, personnel will be able to create scans that will provide all of the accuracy and precision necessary. Readouts and displays for the AMPDXA instrumentation will be specifically designed to provide useful (real-time) feedback information to both the astronauts and the ground-based physician monitoring team (as permitted by the mission dynamics).

Current bone and muscle mass measurements (via conventional DXA or ultrasound) are regional averages that obscure structural details. Since the mechanical consequences of lost bone and muscle are reflected in the structure, an absolute determination of skeletal mechanical competence is needed to supplement the loss measurements. Engineering properties of the bones can be derived from DXA-generated BMD data. Our method derives geometrical measurements from the BMD images. From such images, we extract BMD profiles at important skeletal locations (e.g., proximal shaft and femoral neck). Key properties measured and derived from these profiles include the BMD, the subperiosteal width, the section modulus (related to strength), and the cortical dimensions.

Under the original proposal effort, FY 1998-2000, the AMPDXA project made significant progress in several key areas: (1) instrument development, (2) algorithm development for BMD

image extraction and structural analysis, and (3) bone reconstruction and modeling techniques. During the FY 1998-2001 period, both a full-sized (one-meter source-to-detector distance) Laboratory Test Bed and a system for human testing were constructed. This system was initially called the Clinical Test System in previous reports, but is now called the Human Test Bed to better reflect the nature of the human testing to be performed on the system. The Laboratory Test Bed was utilized to verify principles and theoretical predictions and demonstrate that the AMPDXA techniques worked and produced results with the expected precision. The Human Test Bed has even greater precision.

The Human Test Bed incorporates high-precision, rotational and translational stages to provide the scanning capability to carry out qualification tests on human subjects. Since the Human Test Bed is designed to operate only on Earth, the table, gantry, and associated equipment were not built to the size and mass requirements of an AMPDXA unit for spaceflight. In fact, the unit was built on a used CT scanner. Employing used equipment for some of the structural elements and rotating parts and machinery allowed critical resources to be focused on the information extraction and analysis issues leading to human testing.

The image extraction capability of the AMPDXA is not only the BMD image higher resolution, but also the mass distribution in a projected thickness of a femur slice contains much more structural detail than conventional DXAs. The high frequency content of the BMD spatial projections are reproducible and provide information on the bone's microstructure. Using multiple projections (three or greater) about the bone axis allows structural properties (e.g., bending strength) to be obtained independent of patient position. Initial experimental measurements with different sets of three projections showed that the principal moments of inertia could be determined within three to four percent. Additional projections (above three) reduce this number further. Our original experimental system also had some known nonlinearities, which have since been removed, and our error in the three-projection estimation of moments is less than one percent.

For the 2002 period, we are focused on resolving certain key issues about the AMPDXA and then successfully using the AMPDXA for human testing. These key issues include: (1) unequivocal demonstration that multiple projection technology improves BMD accuracy and collects structural details, (2) the structural details can be converted into bone reconstruction models that preserve mechanical behavior, (3) the reconstruction models can be utilized to predict risk of fracture, (4) soft tissue can effectively be distinguished from bone and decomposed into fat and muscle, (5) data can be collected reliably and repeatedly on human subjects using the Human Test Bed, and (6) the Human Test Bed can be utilized in research studies on bone and muscle loss.

A dual monitor computer system currently operates the AMPDXA as well as records and displays image maps (bone mineral density, muscle, etc.) at near real-time speeds. The main screen presents two views of the BMD images of a human hip collected from a live human test subject. The major accomplishments during the period include reverification and calibration of the Human Test Bed after the move, improving the AMPDXA operational software (providing full documentation and configuration control), refining the image extraction algorithms, demonstrating the Human Test Bed's accuracy and repeatability, and human imaging. Approval for our human testing protocol has been granted by the Institutional Review Board at the Johns Hopkins Medical Institutions.

The AMPDXA project has many implications for future research and development. The AMPDXA, as described above, has direct application to risk reduction in NASA's Critical Research Path. The AMPDXA is capable of real-time monitoring of bone and muscle loss at extremely high precision. Since the results are patient-specific and not tied to volumetric averages and statistical norms, the AMPDXA is a very useful tool for monitoring the effectiveness of countermeasures as well as determining risk of fracture under various loading conditions and activity scenarios. The AMPDXA also appears to be a natural adjunct to Earth-bound research on the effects of aging and disuse on bone integrity. It could also be used as a routine screening tool for osteoporosis and as a monitoring instrument for osteoporosis drug therapy.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Brian L. Davis, Ph.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Design and Validation of a Dynamic Exercise Countermeasure Device

Project Executive Summary

The objective of this study is to design and develop an exercise device that primarily counteracts microgravity induced bone loss and muscle atrophy. Secondary benefits will include alleviating some of the problems associated with vestibular and cardiovascular adaptations to microgravity. This project falls under the technology development designation of the NSBRI program. This three-year project is divided into three phases. In Year 1 we collaborated with Foster Miller Inc., a company that has expertise in the design of both lightweight structures and vibration isolation methodology, to construct a device that permits dynamic jumping exercise in microgravity and that is suitable for the International Space Station. In Year 2 we have conducted ground-based studies in the exercise device to verify that muscle activation patterns are not compromised by the exercise device and that limb loading data, as measured by forces experienced under the feet during jumping, are in the range that is believed to maintain the integrity of bone. In Year 3 we will confirm the efficacy of the exercise device in true microgravity through KC-135 experiments.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Richard H. Maurer, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Neutron Energy Spectrometer Flight Experiments

Project Executive Summary

As a product of our previous NSBRI funding during FY 1998-2001 we had designed and fabricated an engineering prototype neutron spectrometer that was flown on F-15 and F-18 aircraft flights from NASA Dryden Flight Center. The spectrometer consists of both low and high energy subsystems. The detection of low energy neutrons (0.025 eV-1 MeV) is accomplished using a conventional helium three gas tube and includes thermal and epithermal neutrons. The detection of high energy neutrons (5-800 MeV) is achieved using a 5 mm thick lithium drifted silicon solid state device. Both low and high energy spectrometers underwent ground based evaluation and calibration using radioactive sources and accelerator facilities.

We took data up to 39,000 feet in April 2000 on ascent (the plane was to fly at a planned 40,000 feet cruise level) when we experienced a corona breakdown in the high voltage supply systems for both detectors. The instrument was returned to APL so that significant re-design of the detector high voltage power supply systems could occur. The high voltage electronics of the low energy detector were all co-located in the same compact volume so that it could all be adequately potted for protection against breakdown at high altitude in the corona region. In contrast, a hermetic enclosure was designed and fabricated from just two pieces of aluminum, a three dimensional box and its top, for the high energy silicon detector and its associated high voltage electronics. The aluminum top was grooved to accommodate a vacuum seal O-ring. The design and fabrication of these improvements to the spectrometer took place between February and May 2001. The complete instrument was re-qualified in a medium sized vacuum chamber at APL during late May and early June 2001 to a maximum altitude of 61,000 feet with a substantial dwell time of two hours at 56,000 feet to provide almost 50 percent margin on the aircraft flight altitude of 40,000 feet. The instrument was shipped to NASA Dryden on June 15, 2001.

The neutron spectrometer was flown August 13-14 in a pod under the wing of an F-18. Both flights were successful and the data is being analyzed. A third successful flight in the same pod under the fuselage of an F-15 was executed in October 2001. The main positive result from the three flights is the verification of our engineering design and qualification and not the limited data obtained. The value for our hardware is the proven approach to handle the high voltage at high altitude in the corona region that will be employed for a future balloon flight during which scientifically interesting data will be acquired.

In January 2000 we were notified that our proposal titled "Development of a Neutron Spectrometer to Assess Biological Radiation Damage Behind Spacecraft Materials" submitted in March 1999 in response to NASA NRA 98-Heds-05 would be funded for a period of 3.5 years from May 2000 to November 2003 at a level of \$90,000 per year for a total of \$315,000. Originally, our primary responsibility under this grant was to support Lawrence Berkeley Laboratory (LBL) personnel in the evaluation of spacecraft structural and shielding materials by supplying a version of the neutron spectrometer compatible with ground-based accelerator research. The first and only collaborative experiment was carried out in January 2001. Lack of NASA funds for beam time have cancelled heavy ion experiments at the Brookhaven Alternating

Gradient Synchrotron (AGS) originally scheduled for September 2001 and March 2002. We fabricated a detector stack system specifically for these accelerator experiments during the summer of 2001. We have verified its successful operation at Columbia University's RARAF in November 2001 and will now proceed with our own spacecraft shielding experiments using proton and heavy ion beams during FY 2002.

The major effort in detector evaluation in FY 2000 was a series of experiments at the Los Alamos Neutron Science Center (LANSCE) to measure energy deposition in the 5mm thick lithium drifted silicon detector by neutrons with an energy range from 20-800 MeV. The experiments were performed by integrating our 5mm silicon detector with the LANSCE time-of-flight neutron spectrometer on the 90 meter beam line to give simultaneous measurements of the incident neutron energy (LANSCE fission chamber) and energy deposited in our detector. Energy depositions of up to 150 MeV were seen from the up to 800 MeV incident neutrons in our 5mm detector. A major effort during FY 2001 was the extensive analysis of the LANSCE data and the successful development of a response function for the detector between 20 and 600 MeV. We verified our procedure for deducing the response function by successfully comparing its outcomes with the LANSCE beam monitor data in a blind experiment. By using several thicknesses of polyethylene shields in these experiments we began gathering experimental data on the effectiveness of this material as a high energy neutron moderator. The report of these shielding experiments was published in the December 2001 volume of the IEEE Transactions on Nuclear Science. The development of the silicon detector response function was submitted to the journal Radiation Research in February 2002. We have recently completed a set of experiments at Columbia University RARAF using mono-energetic neutron beams between 10-20 MeV to extend the silicon detector response function down to those energies. These experiments included a Cesium Iodide scintillation crystal enclosing the thick silicon detector to discriminate against background gamma rays prevalent in the target room.

The modeling component of this research program occurred on a continuous basis between FY 2000 and FY 2002. We concentrated on modeling the high-energy channel from detailed cross sections of the basic neutron-silicon interactions using state-of-the-art computer codes. There are four reasons to develop this advanced modeling capability: 1) to assess the accuracy of the codes themselves to predict energy deposition in a silicon detector (by comparison with experimental data); 2) to use the codes in understanding the experimental results; 3) to determine whether the codes can be used to calculate the shielding and scattering effects of the instrument packaging and surrounding environment (structure or atmosphere); 4) to assess the ability of the codes to supplement the determination of the instrument response function at interpolated and extrapolated energies (since it is impractical to test at intervals of 10 MeV for the whole energy range). We have found that the GEANT4 code originally developed at CERN is the easiest to use, is maintained in a timely fashion by its developers and reproduces our RARAF (2-20 MeV) energy deposition data reasonably well.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Richard S. Potember, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Real-Time Analysis of Biomarkers and Countermeasures Using a Miniature Time-of-Flight Mass Spectrometer

Project Executive Summary

Original Aims of the Project

- To design, develop and test a fast, portable gas chromatograph – time-of-flight mass spectrometry (GC-MS) system for future human spaceflight applications. It will provide complementary information to the MALDI method.
- To demonstrate that the miniature TOF system is capable of detecting and quantifying different biomarkers that appear in serum or urine during space flight. Detection and quantification of critical biomarkers using the miniature TOF technology will allow real-time monitoring of damage on-orbit, and the mass spectrometer can also be used to study the effectiveness of countermeasures in spaceflight. The results of this effort should be comparable to measurements made in a clinical laboratory facility using established assays.
- To validate that the miniature time-of-flight mass spectrometer is an important diagnostic tool that can be applied to measure important bone biomarkers and the effectiveness of applied countermeasures in human urine and serum samples.
- To compare standard methods of hormone analyses for melatonin and cortisol to that of the Miniature Mass Spectrometer. The development of online methods for monitoring and assessing the status of circadian organization is listed as one of the five primary themes for the Human Performance Factors, Sleep and Chronobiology Team.
- To develop sampling and sample preparation techniques that enable the MALDI TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids with very low error rates.

Key Findings

We have designed and built a new miniature - mass spectrometry (GC-MS) system with a 3-inch analyzer for human spaceflight applications. This miniature instrument will provide new capabilities in the area of sampling, sample preparation, rapid quantitation of biomarkers and it will allow us to apply our technology to other space based problems such as monitoring the spacecraft environment for chemical and biological contaminants.

We have also completed our initial studies on melatonin. In year three we will test melatonin in urine samples at this concentration level.

One of the specific aims of this project is to develop sampling and sample preparation techniques that enable the MALDI TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids (urine, blood,

breath) with very low error rates. In year two, we reviewed several protocols for urine sampling and we have chosen a procedure to conduct measurements for year three.

Impact of these finding on technology objectives

We are developing and testing a small, efficient time-of-flight mass spectrometer coupled to a miniature gas chromatograph to rapidly identify important biomarkers and countermeasures for human space exploration. We are using the time-of-flight mass spectrometer to evaluate critical biomarkers and countermeasures that are indicators of bone loss, oxidative stress and the human sleep cycle associated for extended space travel.

Mass spectrometry is a technique for determining the masses of molecules and specific fragmentation products formed during vaporization and ionization. From detailed analysis of the mass distribution of the molecule and its fragments, molecular identification is accomplished. These molecular measurements can be carried out at the attomole (10^{-18} mole) level of material using specialized laboratory-based instruments. The combination of specific molecular identification and extreme sensitivity makes mass spectrometry one of the most powerful analytical laboratory tools yet developed for detection and identification of chemical and biological substances.

Proposed Research for Year Three

(1) Miniature Mass Spectrometry System

In year three, we will complete testing and evaluation of the new instrument system. We will use the results of this aspect of the project to make a recommendation as to the specific type of instrument that should be built in a follow-on program.

(2) Measurement of Oxidative Stress using TOF Mass Spectrometry

In year three, we will complete the study of oxidative stress biomarkers in urine samples.

(3) Zolendronate: a Countermeasure to Bone Loss in SCI Patients

In year three, we will analyze urine samples for zolendronate and related by-products.

(4) Assessment of Circadian Status Using the Miniature Mass Spectrometer

In year three we will analyze the urine samples from Dr. Kenneth P. Wright Jr. for the excretion of the melatonin metabolite, 6-sulphatoxymelatonin.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Yi-Xian Qin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	A Non-Invasive Scanning Confocal Ultrasonic Diagnostic System for Bone Quality

Project Executive Summary

The bone loss which parallels extended space missions represent serious threat to astronaut health, both during flight and on return to gravitational fields. Early diagnosis of osteopenia would enable prompt treatment and thus dramatically reduce the risk of fracture. The goal of this project is to develop a new technology for monitoring bone quality of humans during long-term space missions and on Earth. This will lead to a better understand of the progressive adaptation of bone loss in astronauts subject to microgravity and aging populations, and the ensuing musculoskeletal complications such as osteoporosis. Results of the joint Russian/US studies of the effect of microgravity on bone tissue demonstrated that bone loss proceeds at an average rate of 2 percent per month, ranging from no loss in the area of upper skeleton to as much as 14-20 percent loss in the skeleton of the lower body following a 14.5-month long mission. While these results are detected only when astronauts returned to Earth, the rate of bone loss *during* space mission is still unclear.

Currently funded by the NSBRI, we are able to develop a scanning confocal acoustic diagnostic (SCAD) system capable of generating acoustic images at the regions of interest (e.g., in the human calcaneus). This portable SCAD system is capable of generating non-invasive, high-resolution ultrasound (US) attenuation and velocity maps of bone, and thus determining the relationship between ultrasonic specific parameters and bone mineral density (BMD), and bone strength and bone's physical properties (i.e., stiffness and modulus). This system is relevant not only for ground-based determination of bone's physical properties, but can effectively be used in the space environment for determining even subtle changes in density and strength during extended flights. In this study, we plan to develop a 2-D ultrasound scanning system, and validate the structure and density information, detected by SCAD, using μ CT and mechanical testing methods in *ex vivo* animal models, as well as correlating to *in vivo* DEXA data derived from humans. The system will thus contribute to monitor degree and risk of bone loss in space and Earth, as well serve as a major step towards clinic usage as an early diagnostic of osteoporosis. There are proposed a series of four original specific aims (S.A.): (1) *Develop a scanning confocal acoustic diagnostic system for non-invasively mapping wave velocity and attenuation in bone;* (2) *Determine an interrelationship between ultrasound determined parameters, i.e., velocity and attenuation, and micro architectural parameters in a quantitative manner;* (3) *Develop a practical SCAD system for determining bone quality properties with quantified bone mass reduction;* and (4) *Map and monitor special directional and orthotropic strength of bone to predict BMD and structural modulus in vivo using the SCAD, and correlate these measurements to DEXA results.*

During this award year (2002-2003), the research team focused on continuing technology development of SCAD system (S.A.1 and 3) and validation between SCAD determined acoustic parameters and bone quality data (S.A.2). Human cadaver and in vivo subject testing were also initiated.

Technology Development

A system design, including hardware and software, of an experimental prototype was established which includes acoustic, electrical, control and mechanical components. As an important step towards a prototype for human testing, a 2-D SCAD system has been built including converging ultrasonic transducers, micro-controller controlled 2-D scanning stages, ultrasonic wave generator, low noise amplifier, and real-time analog/digital (A/D) transformer. A special designed controller is developed and synchronizes digital signals in acoustic wave, scan automation, and A/D transform. This micro-controller guided acoustic scanning technology greatly reduces the scan time, e.g., it requires only approximately 3-4 minutes for a 40x40 pixel array in the region of interest to form images with 0.5~1 mm resolution. Ultrasonic attenuation and velocity images are obtained and calculated, e.g., gray scale or virtual color mapping.

SCAD as noninvasive modality for animal bone quality assessment: The ability of SCAD in non-invasively evaluating bone quality and quantity was tested in a large group of ex vivo bone samples. Trabecular samples were prepared as 1x1x1 cm cubes, which were harvested from sheep femoral distal condyle. These sheep were previously under a mechanical stimuli protocol for 1~2 years and identified distinct bone mineral density using dual-energy X-ray absorptiometry (DEXA). All bone samples were mechanically tested by direct force-deformation in orthogonal directions, i.e., longitudinal, medial-lateral, and anterior-posterior, using a MTS universal test machine. The central plane of the samples was scanned with ultrasonic attenuation and velocity using SCAD system. The results of ultrasonic attenuation and velocity were correlated with mechanical moduli of the sample. While using a single transmitted ultrasound signal, there were weak correlations between measured BUA and micro-CT determined osteo-parameters, e.g., BMD ($R^2=0.28$), porosity ($R^2=0.28$), trabecular thickness ($R^2=0.04$) and trabecular space ($R^2=0.56$), as well as average modulus ($R^2=0.40$). These correlations were significantly improved using the SCAD system. Using SCAD system, μ CT and mechanical testing, new constitutive relations were derived using linear regression correlations in the results, which predict BMD and bone stiffness as the functions of acoustic parameters using combined BUA and UV as well as a series of rational constants. Strong correlations are observed between SCAD determined BUA and micro-CT determined parameters, i.e., BMD ($R^2=0.76$), porosity ($R^2=0.61$), structural mode index ($R^2=0.86$), and average modulus ($R^2=0.71$).

SCAD used for human calcaneus bone quality assessment: The feasibility of SCAD assessment for bone quality in the real body region, which include soft tissue, cortical bone and different surface morphology, is evaluated in cadaver calcaneus. 19 human calcaneus, harvested from cadavers with ages 66~97 have been imaged. BUA and ultrasound velocity determined from region of interest (ROI) have been performed. Bone samples were further tested for structural and strength parameters using μ CT and mechanical testing in the extracted cylindrical samples (10 mm in diameter and 20 mm in medial-lateral length) from ROI. Strong correlations were found between BUA and bone volume fraction (BV/TV) ($R^2 = 0.76$), and between UV and bone's modulus ($R^2 = 0.53$). The correlations are significantly improved ($R^2 > 0.64$) using combined parameters of BUA and UV in linear regression which ultrasound images determined parameters predict structural, e.g., structure morphological index (SMI) ($R^2 = 0.86$), and strength modulus ($R^2 = 0.64$).

These results suggest that high-resolution acoustic mapping is capable of predicting calcaneus bone quantity and quality non-invasively. Structural property parameters of trabeculi, e.g., BMD and BV/TV, is better represented by BUA, while ultrasonic wave velocity has a strong agreement with bone's strength property, e.g., modulus. Ultrasonic imaging has shown the great potential to be used as *in vivo* diagnostic modality for assessing skeletal disorder, i.e., osteoporosis.

Pilot study for human bone quality assessment at large critical site, e.g., proximal femur, using SCAD and DEXA: To explore the potential of using SCAN to detect ultrasound bone image in the hip, human cadaver hip region is tested using an experimental SCAN system. This can evaluate the feasibility of ultrasonic assessment of bone quality *in situ*, which includes cortical bone and different surface morphology. The acoustic confocal region converged in the middle of the coronal plane of the hip with a focal zone approximately 0.5 mm in diameter in the focal region. Thus, a 2-D scan covers the central bone of the proximal hip. The confocal scan area covers an approximate $100 \times 100 \text{ mm}^2$ with a 0.5 mm increment. The signals transmitted through the bone are processed to calculate the slope of the frequency-dependent BUA (dB/MHz), the ultrasound attenuation (ATT, dB), and the ultrasound wave velocity (m/sec), and to generate BUA, ATT or UBV images. The data demonstrated that SCAD is capable to detect bone tissues in the critical skeletal sites, e.g., hip.

Impact

The results have demonstrated the feasibility and efficacy of SCAD for assessing bone's quality in bone (CRL 4 and 5). With proof of the concept using SCAD in bone quality assessment, we have filed two new technology disclosures through University's Technology Transfer Office. Five journal papers are either published or under review, and approximately 12 conference papers were published, which are directly derived from this work. We have been able to demonstrate that the bone quality is predictable via non-invasive scan ultrasound imaging in the ROI, and to demonstrate the strong correlation between SCAD determined data and μ CT identified BMD, structural index, and mechanical modulus. These data have provided a foundation for further development of the technology and the clinical application in this continuing research.

Research plan for the coming year

In the coming year, the research team will focus on (1) developing a practical SCAD system for determining bone quality properties with quantified bone mass reduction for clinical assessment, (2) assessing human calcaneus bone quality within a selected group, e.g., normal and osteoporosis subjects, to predict BMD and structural modulus *in vivo* using SCAD and DEXA, and (3) developing SCAD system for multiple sites testing, e.g., hip region.

A well-established SCAD system may provide a significant impact in diagnostic of osteoporosis and bone quality. Results may provide insight for addressing the risks of bone loss during prolonged space mission, age-related acceleration of osteoporosis, and monitoring healing of fracture.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Veljko Radeka, Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	Heavy Ion Microbeam and Micron Resolution Detector

Project Executive Summary

Project Aims:

Background and Significance. The use of microbeam provides a unique way to control the number of particles traversing individual cells and localizing the dose within the cell. High energy heavy charged particles transfer their energy to biological organisms through high density ionization and excitation along the particle track even by uniform irradiation. This characteristic microscopically non-uniform dose delivery is expected to induce complex DNA damage and mutagenesis, in contrast to relatively uniform dose delivery in gamma-rays or electron beam irradiation. To investigate the distinct biological effects of heavy ions, especially to determine the effects of occupational and environmental exposure of very low doses of heavy charged particles, for example, since virtually no cells receive more than one traversal of cosmic ray HZE particle in its lifetime in a spaceflight environment, one approach is to select cells with the desired exposures from a randomly irradiated population.

Using conventional track segment irradiation methods and sophisticated ion track detecting techniques, the position of the target cells and the ion tracks can be measured together. However, this conventional approach is not practical because all responses of many cells which do not contribute to the aim of the irradiation experiment must be measured. The alternative is to control each ion hit so that irradiation experiment is not a random Poisson process. A heavy ion microbeam can be used to selectively irradiate individual cells which can be analyzed afterward to determine what changes have occurred to that cell and to its un-irradiated neighboring cells.

Although the characteristic biological effects of heavy ions are supposed to be linked to the induction of high-LET-specific DNA lesions, other pathways of radiation effects are still interesting to consider, and there have been several reports that radiation effects of heavy ions may be transmitted from irradiated cells to neighboring unirradiated cells. A heavy ion microbeam can be used to look for pathways other than DNA damage, e.g., damage to the cell membrane or cytoplasm.

The localized dose delivery of a heavy ion microbeam can be applied to the inactivation of a microscopic region of target organisms, cell surgery technique. Heavy ion beams have been applied to radiation therapy development and radiation biology. For evaluation of radiation risk on mammalian cells, proton and helium ions with energies of a few and several MeV from Van de Graff accelerators are mostly used. However, heavy ion beams from those accelerators cannot be applied to the study because of their poor penetration.

Therefore, we want to design and test a high-energy microbeam apparatus and a micron-resolution solid state detector for space radiobiology studies. In addition, we will develop *in vitro* models relevant to radiation risk using a microbeam capable of delivering individual charged particles to individual cells *in situ*. The system will allow us to critically determine the response of human cells to the single-particle traversals typically encountered in space environmental exposures, avoiding the confounding effect of the Poisson distribution of particle

traversals inherent in conventional exposure systems. During long-term space flight mission, it is estimated that virtually no cell receives more than one *Fe* ion traversal in 3-year Mars mission scenario. Thus the use of the microbeam will aim to produce data for direct input into the analysis of human health risks during long-term space flights exposures involving exposure to low fluences of charged particles.

A single-ion microbeam facility comprises a number of elements arranged to deliver reliably counted numbers of ions to a chosen biological target. The elements are:

- a) a source of ions of the appropriate energy,
- b) a means of limiting the location of the ions to an area less than the area of the target,
- c) a means of locating and moving the biological targets to the beam position,
- d) a means of detecting each ion as it traverses the target, and
- e) a means of shutting off the beam after the arrival of the chosen number of ions.

A principal objective of this project is to develop and demonstrate a high resolution silicon detector, which will be able to determine the position of impact of energetic heavy ions in single cell radiation effects studies to within ~ 1 micron. An additional objective is a conceptual design of a heavy ion microbeam in the energy range up to 3 GeV. The beam will be collimated to $\sigma \sim 10$ -20 microns, to a region of one or very few cells. The microbeam will be implemented in a separate project at the BNL NASA Space Radiation Laboratory (NSRL), previously known as Booster Accelerator Facility(BAF).

These developments will advance significantly the state-of-the art of high energy heavy ion microbeams and of high resolution heavy ion detectors. For the cell studies employing these tools, the necessary infrastructure will include a micro positioning stage with a microscope and auxilliary detectors.

Results:

A new concept for a silicon detector with micron position resolution for heavy ions has been developed. First prototypes have been completed and initial testing performed. Basic design studies for the heavy ion microbeam in the energy range of several hundred MeV (and up to 3 GeV) at the BNL NSRL have been completed. Existing microbeams are all at much lower energies, less than 20 MeV.

Implications:

The micron resolution detector, together with the microbeam, will be able to localize the position of an ion impact within a particular region of the cell. This is essential for studies in space radiobiology.

Synergisms:

Radiation Effects Program, in particular, damage to central nervous system from radiation exposure. The objectives and specific aims of this project have been defined in consultation and with the agreement of the team working on radiation effects studies.

Milestones:

The milestones for the first two years of the project have been completed. In, particular, detector design has been developed, first prototypes completed, readout electronics with position interpolation has been developed, and basic microbeam design parameters have been achieved.

Research Plan for the Coming Year:

In the final year of the project, the processing of the second version of the detector prototype (which is expected to be the final version) will be conducted. Tests of the new detectors will be performed. Design study for the microbeam will be completed.

First half of the third year: Finish the processing of the second version of the prototype detectors.

Second half of the third year: Finish the tests (iron beam) of the new detectors. Produce the required number of detectors for the project.

In addition, a demonstration of the detector with cell samples (i.e., localization of incident single iron ions in particular regions of the cell) will be performed (consistent with the schedule of the heavy ion beam facility at NSRL).

**NSBRI RESEARCH PROGRAM
SPACE MEDICINE**

Doerr, H. K. PI Baylor Development and Testing of a Space-Adapted Human Patient Simulator 207

RESEARCH AREA:	Space Medicine
PRINCIPAL INVESTIGATOR:	Harold K. Doerr, M.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Development and Testing of a Space-Adapted Human Patient Simulator

Project Executive Summary

The Medical Operational Support Team (MOST) was created by the National Space Biomedical Research Institute (NSBRI) and was tasked by the JSC Space Medicine and Life Sciences Directorate (SLSD) to incorporate medical simulation into 1) medical training for astronaut-crew medical officers (CMO) and flight control teams and 2) evaluations of procedures and resources required for medical care aboard the International Space Station (ISS).

The MOST has incorporated medical simulation into Space Medicine Operations by using the Human Patient Simulator[®] (HPS) manufactured by Medical Education Technologies, Inc. (METI, Sarasota, FL). The HPS is located in the Medical Simulation Laboratory (MSL) at Wyle Laboratories (Houston, TX). This MSL is a facility consisting of high-fidelity medical equipment and resources similar to those used aboard ISS. The MSL was developed and configured by the MOST to simulate the environment in which flight surgeons, BMEs (biomedical engineers/flight controllers) and CMOs would mitigate medical events on orbit.

In the past year, the MOST has introduced the MSL to multiple operational groups at JSC as well as the training approaches of medical simulation, in particular, Crew Resource Management (CRM). CRM is a communication system that was developed for the medical environment from communication techniques used in flight simulation, including critical thinking, critical communication and algorithm development. The MOST has begun to teach these concepts in concert with space medical training to both flight surgeons and BMEs. This effort is in line with the MOST's goal to develop a training curriculum for each of these flight controller disciplines in addition to a curriculum for Medical Flight Control Teams (flight surgeon, BME and CMO).

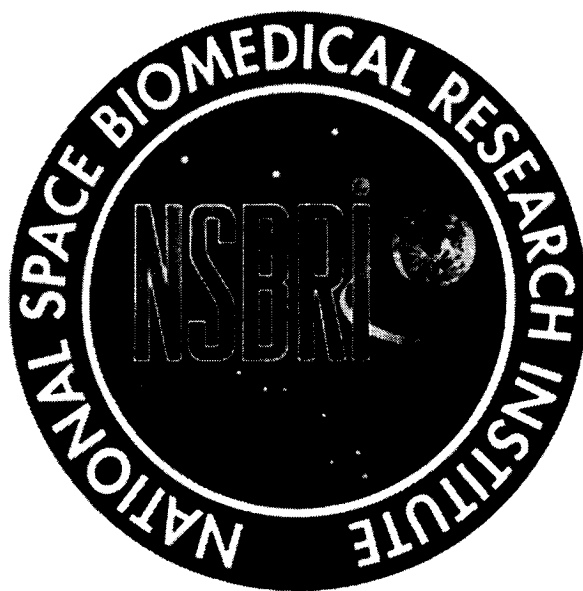
The efforts completed by the MOST in the past year have not been limited to curriculum development. The team has begun collaborating with an industrial leader in ventilation and a prominent military medical institution to conduct a study that would augment the standard of CPR care aboard ISS. In addition, the MOST has contributed to outreach events as demonstrated by its members being selected to be Plenary Speakers at the National Youth Leadership Forum (NYLF) Congress in July 2003. The MOST has also validated skill set training for surgical and non-surgical airway management as well as pneumothorax. The MOST has also developed a training regimen that allows NASA and Department of Defense (DOD) flight surgeons who are assigned for Russian Soyuz spacecraft recovery to administer anesthesia to a returning astronaut/cosmonaut "in the field" should it be necessary following the crew's return from an ISS mission. Recently, the MOST provided a Russian flight surgeon with refresher training in Advanced Cardiac Life Support (ACLS) in preparation for his ACLS exam. Taken together, the MOST has not only followed its intended objectives but has significantly expanded its role to the JSC Space Medicine Community.

The establishment of the MOST by the NSBRI has enabled SLSD to begin augmenting the standard of medical training for medical flight control teams and expand other areas of medical

training. In the coming year, the MOST will continue this effort by not only validating its training curriculums for the designated space flight disciplines but will also implement a Continuing Medical Education credit program for flight surgeons as per the guidelines of the American College of Graduate Medical Education (ACGME) and JSC Space Medical Operations. In addition, the MOST will implement video as an interactive medium to provide refresher training for medical flight control teams.

Appendix D

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



***RESEARCH TEAM REPORTS
FY 2003***

**National Space Biomedical Research Institute
Research Team Reports
FY 2003**

CONTENTS

BONE LOSS

(Not Submitted)

CARDIOVASCULAR ALTERATIONS

HUMAN PERFORMANCE

IMMUNOLOGY, INFECTION & HEMATOLOGY

MUSCLE ALTERATIONS & ATROPHY

NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS

NEUROVESTIBULAR ADAPTATION

NUTRITION, PHYSICAL FITNESS AND REHABILITATION

RADIATION EFFECTS

SMART MEDICAL SYSTEMS

TECHNOLOGY DEVELOPMENT

CARDIOVASCULAR ALTERATIONS TEAM

National Space Biomedical Research Institute

Annual Program Report

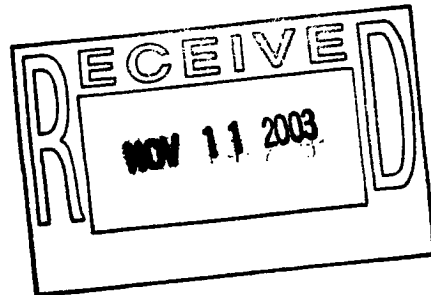
October 1, 2002 – September 30, 2003

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A handwritten signature in black ink, appearing to read "R. J. Cohen". The signature is fluid and cursive, written over a horizontal line.

Richard J. Cohen, M.D., Ph.D.

November 3, 2003

Listing of Team Projects

Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model.

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Integrative Cardiac Myocyte Model: Ion Channels, Ca and Contraction

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Microgravity and Circadian Cardiovascular Function

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Cardiovascular Effects of Simulated Microgravity in Man (1)
Effects of Space Flight on Cardiovascular Stability (2)

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Distributed Simulation of Integrated Human Function

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Circulatory Remodeling with Simulated Microgravity

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Cardiac Unloading: Biologic Mechanisms and Countermeasures for Cardiac Atrophy

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Computational Models of the Cardiovascular System and its Response to Microgravity and Disease

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Integrated Modeling of Cardiac Mechanical and Electrical Function

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Mechanisms of Post-Spaceflight Orthostatic Intolerance

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Development of a Soluble Guanylyl Cyclase Knockout Mouse Model

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Effect of Simulated Microgravity on the Vestibulosympathetic Reflex in Humans

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Mechanics of Cardiovascular Deconditioning

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Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity

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Influence of Gender and Age on Renal and Cardio-Endocrine Responses to Simulated Microgravity

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I. ABSTRACT

During space flight the cardiovascular system undergoes adaptive changes in structure and function in response to microgravity and other factors. While these adaptations appear to be associated with generally adequate cardiovascular performance during conditions of short-duration space flight, they are not appropriate upon reentry into a gravitational environment. The extent of cardiovascular adaptation appears to increase with duration of space flight. The extent and implications of these adaptations for long-duration (months to years) space flight remain largely unknown. Space flight is associated with a movement of fluid from the lower extremity to the thorax and head, a modest decrease in intravascular volume, and a modest decrease in arterial pressure. During space flight the cardiovascular system is not subjected to the stresses associated with changes in posture in a gravitational field. Space flight is associated with, in addition to microgravity, other physiologic stressors such as sleep disruption, confinement and other environmental alterations which may also adversely affect cardiovascular structure and function. As a result of the foregoing factors, space flight results in remodeling of the heart, arterial and venous blood vessels, and the lymphatics. In addition there are alterations in the neural and hormonal control systems.

Adverse effects of space flight on the cardiovascular system: 1) Upon reentry into the Earth's gravitational field, astronauts experience orthostatic intolerance, which limits their ability to function during reentry and after landing and possibly could interfere with the ability of astronauts to egress from the spacecraft under emergency conditions. Currently-used countermeasures, such as oral administration of salt and water prior to reentry and application of anti-gravity suits, do not adequately prevent orthostatic intolerance, especially following long-duration space flight. 2) A number of anecdotal reports suggest that long-duration space flight might lead to an increased incidence of potentially serious heart rhythm disturbances. If space flight does in fact significantly decrease cardiac electrical stability, the effects could be catastrophic, potentially leading to sudden cardiac death. 3) Long-term space flight may lead to a measurable reduction in cardiac mass. It is not known whether these cardiac alterations are reversible and whether they pose a long-term health risk to astronauts. 4) Long-duration space flight may exacerbate previously undetected cardiovascular disease, such as coronary artery disease. 5) Long-term space flight may impair cardiovascular response to exercise.

The objective of the Cardiovascular Alterations Team is minimize these risks using the following approach:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

This approach involves an integrated team effort involving projects ranging from the molecular, cellular, organ system, and whole animal investigations as well as computer simulations.

The Cardiovascular Alterations Team has had two flight projects approved and funded. These are the first NSBRI projects to proceed to flight testing. Of course actual flights experiments have been delayed due to the Columbia tragedy.

The Cardiovascular Alterations Team has developed the alpha-sympathetic agonist midodrine as a countermeasure to the development of post-flight orthostatic intolerance. This countermeasure involves the astronaut taking a single oral dose of this medication at the end of the space flight, and thus does not interfere with cardiovascular physiological function during space flight. In contrast, countermeasures which have been attempted in the past, such as inflight lower body negative pressure interfered with the normal cardiovascular adaptation to microgravity and thus may have impaired cardiovascular homeostasis during flight. The midodrine countermeasure was developed by the Cardiovascular Alterations Team in an integrated fashion involving animal studies, computer simulations of cardiovascular function, and ground-based human studies. The midodrine countermeasure has already been tested under a Supplemental Medical Objective protocol in individual astronauts. One astronaut who had suffered severe post-flight orthostatic intolerance during previous flights, had no such symptoms when using the midodrine countermeasure.

The Cardiovascular Alterations Team has demonstrated for the first time that the simulated microgravity (using a 16 day head down tilt bed rest model) alters cardiac electrical processes. Cardiac electrical function was measured using Microvolt T-Wave Alternans testing – a technology developed under NASA and NSBRI sponsorship. A significant increase was found in the level of microvolt T-wave alternans in these studies suggesting that microgravity exposure may increase susceptibility of the heart to ventricular tachyarrhythmias.

The Microvolt T-Wave Alternans technology developed under NASA and NSBRI sponsorship has been rapidly gaining clinical acceptance as a means of identifying patients at risk of sudden cardiac death. Medtronic, Inc has just launched an 1800 patient study (the *MASTER TRIAL*) to evaluate this technology as a means of identifying which cardiac patients may or may not require an implantable cardioverter defibrillator to prevent sudden cardiac death.

II. INTRODUCTION

During space flight the cardiovascular system undergoes adaptive changes in structure and function in response to weightlessness and other factors, such as sleep disruption, confinement and additional environmental alterations. Space flight is associated with a movement of fluid from the lower extremity to the thorax and head, a modest decrease in intravascular volume, and a modest decrease in arterial pressure. In addition, there are alterations in the lymphatic, neural and hormonal control systems. While these adaptations appear to be associated with generally adequate cardiovascular performance during conditions of short-duration space flight, they are not appropriate upon reentry into a gravitational environment. Furthermore, the extent of cardiovascular adaptation appears to increase with duration of space flight, and the magnitude and implications of these adaptations for long-duration space flight (that is, months to years) remain largely unknown.

Specific adverse effects of space flight on the cardiovascular system include:

1) **Impaired Cardiovascular Response to Orthostatic Stress** Upon reentry into the Earth's gravitational field, astronauts experience orthostatic intolerance, which limits their ability to function during reentry. In many cases, the orthostatic intolerance is sufficiently severe that astronauts cannot stand erect for some time after landing and thus may interfere with the ability of astronauts to egress from the spacecraft under emergency conditions. Upon reentry into a gravitational field blood pools in the dependent veins and arteries which leads to reduction in preload to the heart resulting in a decrease in stroke volume, cardiac output and arterial blood pressure. Factors involved in the development of orthostatic intolerance may include structural and functional adaptations of the heart and arterial and venous blood vessels and lymphatics, alterations in volume control mechanisms, alterations leading to an inadequate or defective neural and hormonal regulatory response, alterations in local vascular reactivity, and mechanisms controlling regional distribution of blood volumes and flows. Factors such as age, gender, genotype, as well as occupational, physical training and dietary history may affect individual susceptibility to the development of post-flight orthostatic intolerance. Currently-used countermeasures, such as oral administration of salt and water prior to reentry and application of anti-gravity suits, do not adequately prevent orthostatic intolerance, especially following long-duration space flight.

2) **Occurrence of Serious Cardiac Dysrhythmias** A number of anecdotal reports suggest that long-duration space flight might lead to an increased incidence of potentially serious heart rhythm disturbances. If space flight does in fact significantly decrease cardiac electrical stability, the effects could be catastrophic, potentially leading to sudden cardiac death. It will be important to determine the mechanisms underlying this phenomenon in order to develop appropriate countermeasures. Potential mechanisms that might lead to reduction in the stability of the electrical substrate include electrolyte changes, changes in the neural and hormonal milieu, and alterations of cardiac myocytes, myocyte connectivity and extracellular matrix resulting from space flight. These alterations may in turn lead to changes in cardiac conduction and repolarization processes which predispose to sustained rhythm disturbances.

3) **Diminished Cardiac Function** Long-term space flight may lead to a measurable reduction in cardiac mass, probably associated with cardiac remodeling. It is not known whether these cardiac alterations are reversible and whether they pose a long-term health risk to astronauts. Factors that may be involved in alterations in cardiac function include changes in myocyte number, size, and geometry; changes in myocardial matrix and microvasculature; alterations in myocyte and organ-level mechanical performance; changes in cardiac gene programming; stimuli and signals that lead to loss of cardiac mass and remodeling; factors affecting reversibility and recovery from these alterations.

4) **Manifestation of Previously Asymptomatic Cardiovascular Disease** Long-duration space flight may exacerbate previously undetected cardiovascular disease, such as coronary artery disease. Little is known about what conditions of space flight may tend to make pre-existing disease symptomatic or accelerate the progression of the underlying disease. Also, we do not know what procedures should be applied to screen astronauts for the presence of asymptomatic cardiovascular disease prior to long term missions

5) **Impaired Cardiovascular Response to Exercise Stress** Long-term space flight may impair cardiovascular response to exercise. Current inflight exercise programs appear adequate to maintain aerobic exercise capacity.

The Objectives of the NSBRI Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

The Critical Issues faced by the Cardiovascular Team in addressing the Critical Risks include:

- Development of Suitable Experimental Models
- Development of Suitable Experimental Approaches
- Development of Mathematical and Computer Models
- Development of New Cardiovascular Technologies
- Addressing the Multiple Conditions Imposed by Space Flight
- Countermeasure Development Issues
- Determinants of Individual Susceptibility to the Adverse Cardiovascular Effects of Space Flight
- Development of Spin-off Technologies to Benefit Clinical Medicine on Earth
- Development of a Space Flight Database

III. Team Structure and Design

The overarching intentions of the Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

The program's overall strategy is dictated by the relevant risks: The following risks are deemed to be high priority and are the focus of the team's efforts:

- Impaired Cardiovascular Response to Orthostatic Stress (14)
- Occurrence of Serious Cardiac Dysrhythmias (13)
- Diminished Cardiac Function (15)

The remaining two risks are deemed to be of lower priority:

- Manifestation of Previously Asymptomatic Cardiovascular Disease (16)

- Impaired Cardiovascular Response to Exercise Stress (17)

While some of the projects do address some aspects of these two risks, no one project principally addresses these risks.

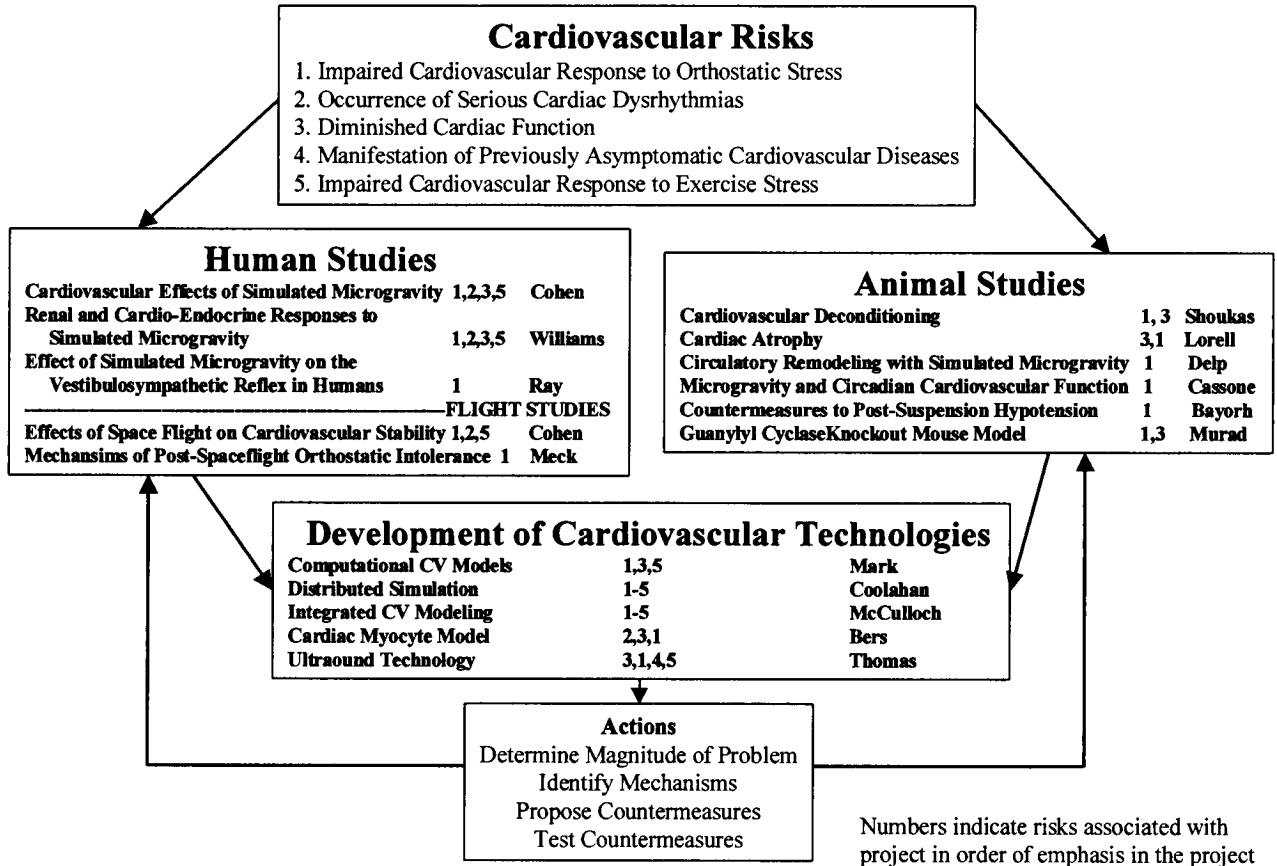
Additional non risk-based goals include those associated with the development of new cardiovascular technologies for use in space and for earth based applications.

The current program is summarized in the Figure.

The Cardiovascular Alterations Team was enlarged by having had three projects (Bers, Coolahan, McCulloch) transferred from the former Integrated Physiology Team. This transfer was the result of an NSBRI management decision that it was better for the computer modeling and simulation projects to reside in specific teams rather than to be grouped in a separate Integrated Physiology team. Mark's cardiovascular modeling project has been part of the Cardiovascular Alterations Team since its inception and very well integrated into the team's activities. Also, Dr. Murad's project was also recently incorporated into the team. The Cardiovascular Alterations Team now involves 16 projects (see Figure). While we have always attempted to have horizontal communication across all team projects, with the growth of the team to 16 projects we have decided to organize the projects into groups of Human Studies, Rodent Studies, and Cardiovascular Technologies.

FIGURE

Cardiovascular Alterations Team



IV. TEAM ACCOMPLISHMENTS

Overview

Below we provide a summary of the accomplishments of the Cardiovascular Alterations Team, followed by reports of progress for each individual project.

We are quite pleased that the Cardiovascular Alterations Team has had two flight projects approved and funded. These are the first NSBRI projects to proceed to flight testing. Of course actual flights experiments have been delayed due to the Columbia tragedy.

The Cardiovascular Alterations Team has developed the alpha-sympathetic agonist midodrine as a countermeasure to the development of post-flight orthostatic intolerance. This countermeasure involves the astronaut taking a single oral dose of this medication at the end of the space flight, and thus does not interfere with cardiovascular physiological function during space flight. In contrast, countermeasures which have been attempted in the past, such as inflight lower body negative pressure interfered with the normal cardiovascular adaptation to microgravity and thus impaired cardiovascular homeostasis during flight. The midodrine countermeasure was developed by the Cardiovascular Alterations Team in an integrated fashion involving animal studies, computer simulations of cardiovascular function, and ground-based human studies. The midodrine countermeasure has already been tested under a Supplemental Medical Objective protocol in individual astronauts. One astronaut who had suffered severe post-flight orthostatic intolerance during previous flights, had no such symptoms when using the midodrine countermeasure.

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Summary of Progress on Strategy

- Good overall progress has been made on the strategic plan, with solid advances made in each of the three targeted cardiovascular risks as well as in the cross-cutting areas of modeling and new technology development.
- **Impaired Cardiovascular Response to Orthostatic Stress** Countermeasure development is furthest along for this risk with two flight studies approved and initial testing of midodrine in astronauts already conducted under the supplemental medical objective program.

- **Occurrence of Serious Cardiac Dysrhythmias** Evidence for affect of simulated microgravity on cardiac electrical processes now established for the first time. Further work is needed to determine mechanisms, determine the level of risk that may be associated with these changes, and evaluate potential countermeasures. One of the two approved flight studies will provide additional data in this regard in that it includes assessment of cardiac electrical stability pre and post flight.
- **Diminished Cardiac Function** Evidence has been found for diminished cardiac function from cardiac unloading on a whole heart and cellular level. However, rat data concerning decreased cardiac mass during hind limb suspension or short duration flight are mixed. We need better longer term flight data. Dr.s Delp and Shoukas have a project to obtain these data which was being flown on Columbia – this project will be reinstated when flights begin again.
- **Modeling** Modeling effort is very strong, but the Cardiovascular Alterations Team is now over-weighted in this area because it inherited three projects from the former Modeling Team.
- **New Technology Development** Excellent record in developing new technologies
 - Microvolt T-Wave Alternans
(technology for non-invasive assessment of risk of ventricular tachyarrhythmias; this technology has been commercialized and is in widespread clinical use)
 - Cardiovascular System Identification
(technology for non-invasive assessment of closed-loop cardiovascular regulation)
 - Echocardiographic Techniques (in conjunction with Smart Med)
(echocardiographic technologies for improved assessment of cardiac function)
 - Pulsatile G Suit
(a “milking” G-Suit countermeasure to the development of orthostatic intolerance)
 - CV Modeling Techniques
(a variety of modeling techniques for simulating cardiovascular electrical and mechanical behavior from the single cell level up to the whole organism level)

Tactical Plan Summary

- Very good collaborations have formed within the team. Nearly all investigators have been good “team players”.
- Excellent collaboration with Cardiovascular Lab at Johnson Space Center. Now that two flight studies approved, we will be engaging JSC management, astronauts and flight surgeons to a greater degree.

- Collaborations exist with other NSBRI teams, but we need to further encourage cross team collaboration.
- We need to further consolidate and focus modeling effort.
- We need to encourage additional new technology development to enhance countermeasure development and for spin-off earth benefits.

Accomplishment Highlights

Impaired Cardiovascular Response to Orthostatic Stress

- Post-suspension hypotension in rats is associated with increased levels of endothelium-derived relaxing factors and is reversed by inhibitors.
- Explored regulation of the soluble guanyl cyclase-cGMP pathway to determine role it may play in the development of orthostatic intolerance.
- Demonstrated critical role of alpha-1b AR subtype in mediating contractile responses in mesenteric micro-vessels during orthostatic stress.
- Hindlimb unloading in rats diminish contractile function in lymphatic vessels.
- Tail suspension of rats abolishes circadian variation in heart rate but not body temperature.
- Used non-invasive technique of Cardiovascular System Identification to obtain pre-bed rest measures that may predict post bed-rest orthostatic intolerance.
- Demonstrated that bed rest leads to activation of the renin-angiotensin system, potassium loss; dissociation between renin and aldosterone responses to simulated microgravity and up-right tilt.
- Single 5 mg dose of alpha-sympathetic agonist midodrine dramatically improves orthostatic tolerance in male subjects after bed rest.
- Melatonin attenuates sympathetic nerve responses to orthostatic stress in humans.

Occurrence of Serious Cardiac Dysrhythmias

- Bed rest dramatically increases the incidence of microvolt T-wave alternans. First controlled data to demonstrate that simulated microgravity alters cardiac electrical processes.
- Demonstrated that microvolt T-wave alternans is predictive of ventricular tachyarrhythmias in patients with LV dysfunction on an ischemic or non-ischemic basis.

Diminished Cardiac Function

- In heterotopic transplant model, cardiac unloading modifies cardiac performance at the level of the individual cardiomyocyte as well resulting in decreased cardiac mass.
- No significant reduction in cardiac mass detected in rats subjected to 7 days of space flight or hind limb suspended for 28 days (Ray and Delp).
- Hind limb suspension however was found by another team investigator (Shoukas) to reduce cardiac mass and to diminish arterial pressure, heart rate, and contractility baroreflexes.

Modeling and Technology Development (Cross-Cutting)

- Developed detailed model of intracellular processes in single cardiac myocyte.
- Developed three-dimensional electromechanical model of ventricular activity.
- Developed and validated integrated model of cardiovascular function, and used to demonstrate that orthostatic intolerance cannot be attributed to any single factor but that alterations in multiple CV mechanisms are responsible.
- Developed computer platform to integrate multiple mechanical and electrical CV models ranging from myocyte to distributed CV system and applying to study of exercise.
- Developed echocardiographic techniques to monitor LV function during space flight.
- Development of pulsatile G suit.

Other Accomplishments

- The CV Alterations Team has had two human flight studies now funded - the first NSBRI flight studies to have made it all the way through the approval process.
- Initial use of midodrine in astronaut who previously had post flight orthostatic intolerance was successful in preventing orthostasis.
- Medtronic is launching an 1800 patient MASTER trial to evaluate microvolt T-wave alternans as a means to identify patients requiring implantable cardioverter defibrillators.
- Accelerating clinical use of microvolt T-wave alternans testing to reduce incidence of sudden cardiac death.
- The CV Alterations Team has been very productive publishing multiple articles in premier journals including:
 - The Lancet
 - Nature
 - PNAS

- Circulation
- Circulation Research
- Journal of the American College of Cardiology
- Journal of Physiology
- Journal of Applied Physiology
- American Journal of Physiology
- Heart
- Hypertension
- Journal of Cardiovascular Electrophysiology
- Journal of Clinical Electrophysiology

INDIVIDUAL PROJECT SUMMARIES

POSSIBLE COUNTERMEASURES TO POST-SUSPENSION HYPOTENSION IN THE HEAD-DOWN TILT RAT MODEL

Principal Investigator: Mohamed A. Bayorh, Ph.D.

Specific Aims

- **To evaluate the mechanism(s) involved in the hypotension observed following release from suspension in the Sprague-Dawley rats, especially the roles of prostacyclin and nitric oxide.**

Accomplishments/Findings

- We have demonstrated that post-suspension hypotension is associated with increased levels of endothelium-derived relaxing factors, such as nitric oxide and prostacyclin in both male and female rats (Bayorh *et al. J. Grav. Physiol.* 8(2):77-83, 2001; Prostagl. Leukotr. Essent. Fatty Acids, 66(5-6): 511-517, 2002). A major observation was that in male rats, the rise in prostacyclin was twice that of females, while in female rats, the increase in nitric oxide was double that of males. Thus, suggesting a gender difference in the mechanisms associated with the reduction in blood pressure. Furthermore, in female rats, (Eatman *et al. Prostagl. Leukotr. Essent. Fatty Acids*, 68(3):197-205,2003) we observed that the levels of prostacyclin during post-suspension remained unaltered in aortic rings but were significantly elevated in carotid arterial rings.
- Also, we found that specific inhibitors of nitric oxide and prostacyclin synthases reverse the reductions in blood pressure post-suspension (Bayorh *et al. Clin. Exper. Hypertension*,23: 611-622,2001; Eatman *et al. Clin. Exper. Hypertension*, 25(1): 35-48, 2002).
- Using a ribonuclease protection assay, PCR and immunoblotting analysis, we also investigated the regulation of cyclooxygenase (COX) and nitric oxide synthase (eNOS, iNOS) expression in kidney and heart tissues following suspension. In the kidney and heart, COX-1 mRNA expression tended to increase while COX-2 mRNA expression declined (*manuscript in preparation*).

Research Plans

- To examine the signal transduction pathways involved in the observed post-suspension hypotension.
- To study the role of gender in the post-suspension hypotensive response.
- To examine any alterations in gene expression associated with the post suspension hypotensive response

Countermeasure Development Plans

- To use the specific prostacyclin synthase and/or nitric oxide synthase II inhibitors (U-51605, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT)) to attenuate the post-suspension hypotension response.
- To use these specific inhibitors in combination with a high salt diet to reduce the post-suspension hypotension response
- To use the combination of these specific inhibitors and salt diet with midodrine to attenuate the post-suspension hypotension response.

Collaborations

- Myrtle Thierry-Palmer, Ph.D., Nerimiah Emmett, Ph.D., and Danita Eatman, Ph.D. – NASA Space Medicine and Life Sciences Research Center at Morehouse School of Medicine, Atlanta, GA

INTEGRATIVE CARDIAC MYOCYTE MODEL: ION CHANNELS, CA AND CONTRACTION

Principal Investigator:

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FAX (708) 216-6308
dbers@lumc.edu*

Co-Investigators:

Pieter deTombe, Ph.D. R. John Solaro, Ph.D.

Specific Aims

- **Develop a more up-to-date electrophysiological model of cardiac myocytes.**
- **Incorporate new Ca transport data on SR Ca uptake, release & Na/Ca exchange.**
- Extend the model to include cooperative Ca-dependent contraction and relaxation.
- Implement model in highly accessible computational formats.

Accomplishments

- **Up-to-date electrophysiological model.** 1. We have completed the first major version of our user-friendly computational model of cardiac ion currents and Ca regulation (LabHEART 4.7) and published a manuscript describing it in the *American Journal of Physiology* (Puglisi & Bers). We have made this model freely available for download from our website (<http://www.lumc.edu/physio/bers>). Over 500 people have downloaded the program (in 38 countries) and it has already been used successfully in teaching medical and Ph.D. students. 2. We used this model to determine the relative contributions of two key factors contributing to arrhythmogenesis in heart failure (upregulated Na/Ca exchange and downregulated inward rectifier potassium current, I_{K1}). 3. We have added versions for epi-, mid- and endocardial ventricular myocyte which helped a collaborating group (Dr. McCulloch's) to incorporate our cellular model into their more integrative whole heart model.
- **New Ca transport version of model.** We have updated LabHEART 4.7 model in several ways already. 1. We have further updated ionic currents (e.g. including important characterization and subdivision of transient outward currents, $I_{to,f}$ & $I_{to,s}$; LabHEART 4.9x). 2. We have added the facility that the user can modify the equations that describe the ionic currents and transporters (LabHEART 5.0). 3. We have also overhauled the model (Shannon-model) to include a more appropriate cellular geometry and compartments based on experimental data (including junctional cleft and subsarcolemmal compartments) and used more up-to-date experimentally tested expressions for Ca current, SR Ca release, SR Ca-ATPase and Na/Ca exchange. This major revision is currently being written up for publication and also being ported to a more versatile computational format (from that in which it was developed). 4. We have used this new model to help distinguish the relative importance of 3 factors that contribute to reducing SR Ca content in heart failure: a) reduced SR Ca-ATPase, b) increased Na/Ca exchange function and c) increased diastolic SR Ca leak,

each of which we have measured experimentally. This work will be published in *Circulation Research* in October (Shannon, Pogwizd & Bers).

- **Extend model to include myofilament properties.** 1. In parallel with the above, we have developed a novel cardiac myofilament model that includes realistic representations of the steep cooperative force- $[Ca]_i$ relationship, the length-dependence of myofilament activation and the load-dependence of contraction duration. This used local filament nearest-neighbor interactions and Monte Carlo simulations. 2. This work was written up and published as a full paper in the *Biophysical Journal* (Rice & deTombe). 3. This sort of Monte Carlo simulation is not practical for incorporation into a cellular ion channel-Ca transport model. So, we have developed a novel ODE (ordinary differential equation) version of this model which retains reasonably well the important characteristics. This version should be practical to incorporate into our current ion channel-Ca transport model.
- **Highly accessible computational formats** This has been an ongoing thrust in all of the above aims. 1. LabHEART 4.7 is the prototype in user friendly version of the model for both teaching and for use by other scientists in the field. The subsequent LabHEART versions have retained this focus (and we have even developed a student tutorial guide). 2. Our work in dovetailing our model for incorporation into Dr. McCulloch's whole heart model constitutes another kind of accessibility that is important (but differs from the stand alone LabHEART). 3. Our newer Shannon-model with additional compartments is also currently being developed both ways (flexible for integration in larger scale models, but also for the stand-alone cellular model).

Research Plans

In the final year we will need to complete many of the ongoing modeling efforts, publish manuscript where appropriate and use them in additional ways. Some key aims are to:

- Complete and publish LabHEART 4.9x and 5.0 versions and make them freely available.
- Complete and publish the new Shannon-model, as well as use it to more fully explore how perturbations in conditions (including rate, adrenergic state) alter electrophysiological and Ca handling properties. Additional perturbations are directly related to ongoing studies by Dr. Lorell's group where changes in expression of Ca transport and ion channels that occur upon cardiac unloading can be more realistically simulated.
- Connect the Shannon-model to the myofilament ODE model to allow the first up-to-date model combining ion channels, Ca transport and contractile elements (in both variants of user friendliness).
- Extend our collaboration with the whole heart modeling efforts of McCulloch's group which will allow more direct studies of the acute affects of cardiac unloading (as in weightlessness) can be explored (and then observed altered cellular expression of transporters and channels) can be superimposed to simulate more long-term systemic compensations.

Countermeasure Development Plans

- This particular project is more tuned to providing a computational platform on which to better understand how changes that occur during spaceflight at the more cellular and molecular level can be understood (and intervened upon) in a more integrated framework. In particular the alterations in expression and function with cardiac unloading described by Lorell's group can be incorporated into our computational model (especially when synthesized into the whole heart context by McCulloch's group) to understand why function

is altered and how that may be practically counterbalanced (e.g. by α -adrenergic stimulation or other strategies)

Collaborations

- Our group already included collaboration of investigators at 4 different institutions with complementary strengths (Bers & Puglisi at Loyola University Chicago, deTombe & Solaro at University of Illinois, Chicago, Shannon at Rush Univ, Chicago and Rice at IBM in New York). This has allowed good progress to be made along all of the specific aims originally proposed. Inter-group collaborative relationships have also developed, especially strongly between our group and that of Dr. McCulloch, and that has extended the sphere of expertise and impact of both groups with respect to modeling. Additional interactions between our group and that of Dr. Lorell's has brought some of the biological consequences more clearly into view, and minor interactions have occurred with other modeling and experimentally focused teams.

POSSIBLE COUNTERMEASURES TO POST-SUSPENSION HYPOTENSION IN THE HEAD-DOWN TILT RAT MODEL

Principal Investigator: Mohamed A. Bayorh, Ph.D.

Specific Aims

- **To evaluate the mechanism(s) involved in the hypotension observed following release from suspension in the Sprague-Dawley rats, especially the roles of prostacyclin and nitric oxide.**

Accomplishments/Findings

- We have demonstrated that post-suspension hypotension is associated with increased levels of endothelium-derived relaxing factors, such as nitric oxide and prostacyclin in both male and female rats (Bayorh *et al. J. Grav. Physiol.* 8(2):77-83, 2001; Prostagl. Leukotr. Essent. Fatty Acids, 66(5-6): 511-517, 2002). A major observation was that in male rats, the rise in prostacyclin was twice that of females, while in female rats, the increase in nitric oxide was double that of males. Thus, suggesting a gender difference in the mechanisms associated with the reduction in blood pressure. Furthermore, in female rats, (Eatman *et al. Prostagl. Leukotr. Essent. Fatty Acids*, 68(3):197-205,2003) we observed that the levels of prostacyclin during post-suspension remained unaltered in aortic rings but were significantly elevated in carotid arterial rings.
- Also, we found that specific inhibitors of nitric oxide and prostacyclin synthases reverse the reductions in blood pressure post-suspension (Bayorh *et al. Clin. Exper. Hypertension*,23: 611-622,2001; Eatman *et al. Clin. Exper. Hypertension*, 25(1): 35-48, 2002).
- Using a ribonuclease protection assay, PCR and immunoblotting analysis, we also investigated the regulation of cyclooxygenase (COX) and nitric oxide synthase (eNOS, iNOS) expression in kidney and heart tissues following suspension. In the kidney and heart, COX-1 mRNA expression tended to increase while COX-2 mRNA expression declined (*manuscript in preparation*).

Research Plans

- To examine the signal transduction pathways involved in the observed post-suspension hypotension.
- To study the role of gender in the post-suspension hypotensive response.
- To examine any alterations in gene expression associated with the post suspension hypotensive response

Countermeasure Development Plans

- To use the specific prostacyclin synthase and/or nitric oxide synthase II inhibitors (U-51605, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT)) to attenuate the post-suspension hypotension response.

- To use these specific inhibitors in combination with a high salt diet to reduce the post-suspension hypotension response
- To use the combination of these specific inhibitors and salt diet with midodrine to attenuate the post-suspension hypotension response.

Collaborations

- Myrtle Thierry-Palmer, Ph.D., Nerimiah Emmett, Ph.D., and Danita Eatman, Ph.D. – NASA Space Medicine and Life Sciences Research Center at Morehouse School of Medicine, Atlanta, GA

CARDIOVASCULAR EFFECTS OF SIMULATED MICROGRAVITY IN MAN

Principal Investigator: Richard J. Cohen

Specific Aims

- To apply cardiovascular system identification (CSI) technique to investigate quantitatively and non-invasively alterations in cardiovascular regulation and function during simulated microgravity
- To apply microvolt level T-wave alternans (MTWA) analysis to investigate the impact of simulated microgravity on susceptibility to ventricular dysrhythmias
- To test the effectiveness of midodrine at preventing orthostatic intolerance following exposure to microgravity
- To test the effectiveness of spironolactone at preventing the development of changes in human myocardial electrical conduction properties following exposure to microgravity
- To evaluate the effects of age, gender and sleep deprivation

Accomplishments/Findings

- CSI analysis completed on 29 male subjects undergoing 16 days of 4° head-down-tilt bedrest
- Bedrest impairs cardiovascular autonomic control as manifested by diminished heart rate (HR) baroreflex, total peripheral resistance (TPR) baroreflexes and respiratory induced HR variability
- Developed new CSI technique to separately measure sympathetic and para-sympathetic responsiveness.
- Found that low sympathetic and high parasympathetic responsiveness as measured by CSI pre bed-rest identifies subjects who will develop orthostatic intolerance post bed-rest
- Midodrine effective countermeasure to the development of orthostatic intolerance in bed-rest studies, flight study approved.
- Developed new CSI based technique for measuring total peripheral resistance (TPR) arterial baroreflex and the TPR cardio-pulmonary baroreflex.
- Found marked reduction in TPR arterial baroreflex after bed rest.
- Completed studies on 8 (out of a scheduled 14) pre-menopausal women undergoing 16 days of 4° head-down-tilt bed rest. Data to date indicate that women are much more susceptible to post-bed orthostatic intolerance than men.
- Validated CSI derived measures from computer simulation studies of intact cardiovascular system with reflexes
- Developed technique for non-invasive monitoring of cardiac output from analysis of arterial blood pressure wave-form.
- Demonstrated that bed rest increases incidence of microvolt T-wave alternans (MTWA) indicating that exposure to microgravity may potentially increase susceptibility to ventricular tachyarrhythmias.
- Clinical use of MTWA is rapidly growing to prevent sudden cardiac death
- Medtronic is sponsoring 1800 patient study focusing on use of MTWA to identify patients in need of implantable defibrillators.

- New grant received from DOD sponsored Center for Integration of Medicine and Innovative Technology for development of continuous cardiac output monitoring.
- Flight study just received funding.

Research Plans

- Analyze data on sleep deprivation (data collected on 8 subjects)
- Complete data collection on premenopausal women and evaluate effect of midodrine countermeasure
- Collect data on men over age 50 and evaluate effect of spironolactone as an anti-arrhythmic countermeasure
- Conduct flight study with CSI and MTWA measured pre and post flight and evaluate effect of midodrine

Countermeasure Development Plans

- Evaluate midodrine countermeasure to the development of orthostatic intolerance, in bed rest and flight studies
- Evaluate spironolactone as a countermeasure to development of increased susceptibility to ventricular dysrhythmias

Collaborations

- Dr. Gordon Williams Brigham and Women's Hospital – joint bed rest study
- Dr. Janice Meck, Head Cardiovascular Laboratory at JSC

EFFECTS OF SPACE FLIGHT ON CARDIOVASCULAR STABILITY

Principal Investigator: Richard J. Cohen

Co-Principal Investigator: Janice Meck

Specific Aims

- Apply Cardiovascular System Identification to study flight induced alterations in cardiovascular regulation
- Apply Microvolt T Wave Alternans testing to investigate the effects of space flight on cardiac electrical stability
- Test the effects of midodrine in preventing post flight orthostatic hypotension

Accomplishments

- Funding initiated this past quarter

Research Plans

- Initiating the study.

Countermeasure Development Plans

- Flight test midodrine
- Determine whether space flight alters susceptibility to ventricular tachyarrhythmias

Collaborations

- Dr. Janice Meck, Head of Cardiovascular Lab at JSC
- JSC Project Administration Staff
- Anticipate working with “customers” such as flight surgeons and astronauts as project progresses

DISTRIBUTED SIMULATION OF INTEGRATED HUMAN FUNCTION

Principal Investigator: James E. Coolahan, Ph.D. (JHU/APL)
Co-Investigators: Raimond L. Winslow, Ph.D. (JHU/SOM)
Andrew B. Feldman, Ph.D. (JHU/APL)

Specific Aims

- To develop, at JHU, an experimentally-based computational model of the human ventricular myocyte using cells isolated from tissue biopsies performed in patients; and to develop a finite-element model of the geometry and fiber structure of the human heart using diffusion-tensor imaging data to be collected at JHU, fit by a finite-element model to be developed at the University of California, San Diego (UCSD).
- To develop a distributed simulation of human cardiac function, incorporating a simulation of the human cardiac ventricular cell resident at JHU based on the model discussed above and a simulation of coupled cardiac mechanical and electrical function resident at UCSD, with distributed simulation control based at JHU/APL.
- Working with the NSBRI Integrated Human Function (IHF) team, to select other appropriate cardiovascular system models that can be represented over time using simulations, and integrate them into a distributed simulation of cardiovascular function.
- Again working with the NSBRI IHF team, to select bone and muscle models that can be represented over time using simulations, and integrate them into a multi-function distributed simulation representative of the full IHF simulations that will be needed for long-duration space flight.

Accomplishments/Findings (August 2002 through July 2003)

- Completed development of human ventricular myocyte model (in Winslow lab), which can reproduce the following properties of human ventricular myocyte action potentials: action potential shape; AP duration changes as a function of pacing frequency; and extra-systolic restitution and post-extrasystolic potentiation curves. (Risk #13, critical question 3.01)
- Converted High Level Architecture (HLA)-compliant simulation federation of 2D Hybrid Cellular Automata (HCA) ventricle model with MIT-developed RCVSIM cardiovascular system model to new IEEE 1516 HLA standard (first use of new standard in U.S.).
- Developed an HCA-based scheme for computationally-efficient simulation of cardiac electrical activation in 3D cardiac muscle with arbitrary local fiber orientations and local conductivity tensors (that incorporate fiber sheet structure). (Risk #13, critical question 3.01)
- Completed integration and currently initiating testing of an HLA-compliant simulation of human exercise for the standard astronaut cycle ergometer protocol, incorporating revised version of RCVSIM (MIT), combined skeletal muscle energetics (Univ. of Washington) and whole-body lactate metabolism (Case Western Reserve Univ.) model, and simplified local blood flow and respiratory models (JHU/APL). (Risk #17, critical questions 3.12 & 3.13; risk #28, critical question 8.08; risk #49, critical question 12.01)
- Publications:
 - Coolahan, J. E., Feldman, A. B., and Murphy, S. P., "An Interdisciplinary Approach to Integrated Modeling of Human Systems for Spaceflight," in *Proc., Second Joint Meeting of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society (EMBS/BMES)*, CD-ROM, Houston, TX (Oct 2002).
 - Feldman, A. B., Murphy, S. P., and Coolahan, J. E., "A Method for Rapid Simulation of Propagating Wave Fronts in Three-Dimensional Cardiac Muscle with Spatially Varying

Fiber Orientations,” in *Proc., Second Joint Meeting of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society (EMBS/BMES)*, CD-ROM, Houston, TX (Oct 2002).

- Murphy, S. P., Coolahan, J. E., Lutz, R. R., Saunders, R., Kovalchik, J. G., and Feldman, A.B., “Human Physiology Simulation Integration Using the HLA: ExerFed1516,” in *Proc., 2003 Spring Simulation Interoperability Workshop*, CD-ROM, Kissimmee, FL (Mar-Apr 2003). [selected as 4th-best paper on workshop’s recommended reading list]
- Murphy, S. P., and Lutz, R. R., “A Practical Guide to Implementing a Biomedical Federation Using the 1516 RTI with the C++ Interface,” in *Proc., 2003 European Simulation Interoperability Workshop*, CD-ROM, Stockholm, Sweden (Jun 2003). [selected as 3rd-best paper on workshop’s recommended reading list]
- Coolahan, J. E., “A Collaborative Modeling and Simulation Environment for Space Biomedical Research,” in *Proc., 2003 European Simulation Interoperability Workshop*, CD-ROM, Stockholm, Sweden (Jun 2003). [selected as 5th-best paper on workshop’s recommended reading list]

Research Plans (for third award year, completing on March 31, 2004)

- Publish work done in the Winslow laboratory during the second award year on the development of the human ventricular myocyte model.
- Continue and complete work in the Winslow laboratory on the imaging of human hearts, in order to produce the human heart geometry needed for the full human heart model.
- Continue work on the 3D Hybrid Cellular Automata (HCA) heart model; examine use in conjunction with a simple model for the local action potential to provide activation sequences and relaxation dynamics for driving mechanical models of the pumping of the heart; examine integration with RCVSIM, in a revised version of our Cardiovascular-Ventricular System (CVVS) simulation.
- Continue development of the High Level Architecture (HLA)-compliant human exercise federation, working in collaboration with Drs. Cabrera and Kushmerick, testing and refining the simulation using cycle ergometer data collected in a laboratory environment.

Countermeasure Development Plans

- Analysis of the cycle ergometer exercise protocol currently used by astronauts, using the simulation of human exercise.

Collaborations

- Collaborating with Dr. Roger Mark (MIT), et al, of NSBRI Cardiovascular Alterations team on cardiovascular model incorporation in integrated cardiac-cardiovascular simulation and in simulation of human exercise.
- Collaborating with Dr. Martin Kushmerick (Univ. of Washington) of NSBRI Muscle Alterations and Atrophy team on skeletal muscle energetics model integration into simulation of human exercise.
- Collaborating with Dr. Marco Cabrera (Case Western Reserve Univ.) of NSBRI Nutrition, Physical Fitness and Rehabilitation team on whole-body lactate metabolism model integration into simulation of human exercise.
- Engaged in discussions with Dr. R. Donald Hagan, Ph.D., Exercise Lead, Human Adaptation and Countermeasures Office, NASA Johnson Space Center, to ensure representativeness of exercise protocols being used in simulation of human exercise.

CIRCULATORY REMODELING WITH SIMULATED MICROGRAVITY

Principal Investigator: Michael D. Delp

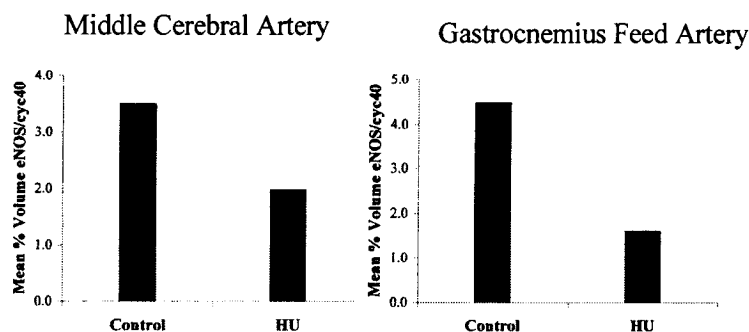
Specific Aims

- To identify early regulatory events leading to hypertrophic remodeling of cerebral arteries in response to hindlimb unloading
- To characterize signaling events leading to atrophy of resistance arteries in the soleus and gastrocnemius muscle in response to hindlimb unloading
- To evaluate the effects of hindlimb unloading on the ability of the lymphatics from different regions of the body to generate and modulate lymph flow, and thus, regulate overall body fluid homeostasis
- To evaluate the effectiveness of using a lower-body negative pressure countermeasure to attenuate hindlimb unloading-induced adaptations in the arterial and lymphatic circulation

Accomplishments/Findings

- Aim #1: One manuscript is in preparation describing structural, vasoconstrictor and vasodilatory alterations induced by hindlimb unloading in the middle cerebral artery. Results demonstrate that basal tone and contractile responsiveness are enhanced, basal and stimulated NO release is diminished, while endothelial nitric oxide synthase protein expression is lower.
- Aim #2: Experiments regarding the time course of hindlimb unloading-induced changes in NOS mRNA and protein expression in the soleus and gastrocnemius muscle arterioles are ongoing. Preliminary results indicate that differences in the remodeling of arterioles from these two muscles differ in regard to changes in NOS expression.

Relative Expression of eNOS Protein after 14 days of Hindlimb Unloading



- Aim #3: Lymphatic contractile function from the mesenteric lymphatics and thoracic ducts have been tested and characterized from control rats, and the results have been published (see below). Furthermore, the effect of hindlimb unloading has been determined to diminish the contractile function in these lymphatic vessels. More

specifically, there is a 50-75% reduction in resting tone of lymphatic vessels, a 30-60% reduction in phasic contraction frequency of the lymph pump, a 60-80% reduction in the strength of phasic contractions of the lymph pump, and a significant reduction in the pressure-sensitive stimulation of the lymph pump. These results not only have important implications for a diminished capacity to maintain appropriate whole body fluid homeostasis, but also may play a major role in the depressed function of the immune system that is associated with habitation in a microgravity environment. These results of simulated microgravity have been presented at the Experimental Biology, Bioastronautics, and the Humans in Space Symposium meetings. A manuscript also is in preparation.

Gashev AA, Davis MJ, and Zawieja DC. Inhibition of the active lymph pump by flow in rat mesenteric lymphatics and thoracic duct. *J Physiol (Lond)* 540: 1023-1037, 2002.

Muthuchamy M, Gashev A, Boswell N, Dawson N, and Zawieja D. Molecular and functional analyses of the contractile apparatus in lymphatic muscle. *FASEB J* 10.1096/fj.02-0626fje, 2003.

Gashev AA, Zawieja DC, and Delp MD. Effects of hindlimb suspension on active pumping of rat thoracic duct and mesenteric lymphatics (Abstract). *FASEB J*. 17: A128, 2003.

Research Plans

We plan to continue work related to specific aims 1-3. These studies continue to provide novel and important information regarding the adaptive responses of the arterial and lymphatic circulations to simulated microgravity.

Countermeasure Development Plans

As indicated under specific aim 4, we proposed to test the effectiveness of lower-body negative pressure in attenuating hindlimb unloading-induced adaptations elucidated in specific aims 1-3 for the arterial and lymphatic circulations. However, in the scientific review of the proposal, the study section recommended that the countermeasure studies be eliminated in favor of the descriptive and mechanistic studies outlined in specific aims 1-3. From this recommendation, as well as the disruption in NSBRI funding, it is unlikely that we will be able to complete specific aim 4. Therefore, it is unlikely that we will be able to complete the countermeasure testing during this funding cycle.

Collaborations

A collaboration between the current project PI (Michael Delp) and the PI of another NSBRI Cardiovascular project, Dr. Chester Ray, was established to determine the effects of microgravity and hindlimb unloading on cardiac mass in rats. This project was funded, in part, by both of the current NSBRI grants to these PIs, and the results have been published.

Ray, C.A., M. Vasques, T.A. Miller, M.K. Wilkerson, and M.D. Delp. Effects of short-term microgravity and long-term hindlimb unloading on rat cardiac mass and function. *J. Appl. Physiol.* 91: 1207-1213, 2001.

Cardiac Unloading: Biologic Mechanisms & Countermeasures for Cardiac Atrophy

*Principal Investigator: Beverly H. Lorell, MD; Harvard Medical School and Beth Israel
Deaconess Medical Center*

Co-Investigator - Michael D. Schneider, MD; Baylor School of Medicine

Specific Aims

To determine functional consequences of cardiac remodeling due to microgravity unloading using the earth-based rodent model of heterotopic transplantation

- Effects on adult cardiomyocyte contractile function and Ca²⁺ regulation
- Regulation of adult cardiomyocyte growth and programmed cell death (apoptosis)
- Identification of human-relevant countermeasures which blunt cardiac atrophy and/or enhance functional cardiac reserve (including alpha-adrenergic agents)

Accomplishments/Findings

I. Heterotopic transplant model: cardiac unloading does depress cardiac performance

Published. Circulation. 2003; 107:1176-82.

- Cardiac unloading directly modifies cardiac performance reserve at level of cardiomyocyte
- Depressed cardiac performance reserve is thus caused by changes in cardiac mass *and* intrinsic cardiomyocyte function
- Mechanism is related to molecular reprogramming and distinct “molecular signature” of Ca²⁺ regulatory genes in the heart
- Changes are related to magnitude and duration of unloading
- Direct implications for study of short vs long-term human spaceflight

II. In vitro studies

Identification of 2 novel pathways for preservation of cardiac mass & function:

- Cyclin-dependent kinase-9
- Anti-aging gene, telomerase reverse transcriptase.

Research Plans

To test the hypotheses:

- Cardiac unloading modifies cardiac adrenergic receptor signaling
- Cardiac unloading stimulates cell cycle entry and programmed cell death, in addition to alterations in myocyte function and morphology

Cardiac Unloading: Biologic Mechanisms & Countermeasures for Cardiac Atrophy

*Principal Investigator: Beverly H. Lorell, MD; Harvard Medical School and Beth Israel
Deaconess Medical Center*

Co-Investigator - Michael D. Schneider, MD; Baylor School of Medicine

Countermeasure Development Plans

I. Heterotopic transplant model:

To test the hypotheses:

- ***Short duration treatment with α -adrenergic receptor agonist enhances cardiac performance reserve: a countermeasure for high work states directly applicable to human astronauts challenged by prolonged microgravity cardiac unloading***
- α -adrenergic receptor stimulation rescues contractile function of unloaded hearts.
 1. *This hypothesis is confirmed* (experiments begun Year II for completion Year III).
 2. In cardiomyocytes from unloaded hearts, α -adrenergic receptor agonist markedly enhances cardiomyocyte contractility.
 3. Biologic mechanisms include augmentation of the cardiac Ca^{2+} transient and improved capacity to correct intracellular acidification.
 4. Accepted for presentation at 2003 HFSA and AHA Scientific Sessions.
 5. Manuscript in preparation now.
 6. **Major synergy with ongoing human spaceflight studies of midodrine as countermeasure for orthostatic regulation.**
- α -adrenergic receptor agonist suppresses induction of cardiac apoptosis in unloaded hearts

II. Transgenic mouse and in vitro model:

To test the hypothesis:

- Gain and loss of function of the anti-aging gene, telomerase reverse transcriptase, modifies the adverse effects of chronic cardiac unloading

Collaborations

- Paul C. Simpson, M.D. VA Medical Center and University of California San Francisco
- Koichi Shimizu, M.D. Brigham & Womens Hospital and Harvard Medical School

Publications - NSBRI Project supported to date

1. Ding B, Price RL, Goldsmith EC, Borg TK, Yan X, Douglas PS, Weinberg EO, Bartunek J, Thielen T, Didenko VV, Lorell BH. Left ventricular hypertrophy in ascending aortic stenosis mice. Anoikis and the progression to early failure. *Circulation*. 2000;101:2854-2862.
2. Ito K, Yan X, Tajima M, et al. Contractile reserve and intracellular calcium regulation in mouse myocytes from normal and hypertrophied failing hearts. *Circ Res*. 2000;87:588-595.

3. Ito K, Yan X, Feng X, Manning WJ, Dillmann WH, Lorell BH. Transgenic expression of sarcoplasmic reticulum Ca²⁺ ATPase modifies the transition from hypertrophy to early heart failure. *Circ Res.* 2001;89:422-435.
4. Minamino, T., Yujiri, T., Terada, N., Taffet, G. E., Michael, L. H., Johnson, G. L., Schneider, M. D. MEKK1 is essential for cardiac hypertrophy and dysfunction induced by Gq. (submitted August 2001; revised November 2001)
5. Ito K, Nakayama M, Hasan F, Yan X, Schneider MD, Lorell BH. Contractile reserve and calcium regulation are depressed in myocytes from chronically unloaded hearts. *Circulation.* 2003; 107: 1176-1182.
6. Nakayama M, Shimizu K, Okoshi K, Schuldt AJT, Swigart PM, O'Connell TD, Simpson PC, Lorell BH. Chronic cardiac unloading enhances the contractile response to α -adrenergic receptor stimulation in isolated myocytes. 2003. In preparation.

Computational Models of the Cardiovascular System and its Response to Microgravity and Disease

Principal Investigator: Roger G. Mark

Specific Aims

- Develop computational models of the cardiovascular system to investigate and evaluate various hypotheses of orthostatic intolerance, and to predict the effects of countermeasures.
- Verify the model using extensive collection of archived data obtained from collaborators.
- Complete, document, and disseminate the model.
- Apply the cardiovascular model to the clinical problem of intelligent patient monitoring with particular emphasis on establishing an enhanced research database of multi-parameter hemodynamic and clinical data from intensive care patients.

Accomplishments/Findings

- *CPR Risk ID 14; Risk Rank 1; Risk Type II, CQ 3.05:*
 1. Validated model against population average and individual subject data.
 2. Through local, national, and international collaborations, established large archive of high resolution hemodynamic data during gravitational stress (including astronaut stand tests and operational tilts and ground-based clinical studies).
 3. Simulations indicate that hypovolemia is the most important contributor to postflight OI, but the hemodynamic responses after exposure to microgravity cannot be explained by a change in one single, yet critical, cardiovascular parameter alone.
 4. Demonstrated the model's ability to simulate the CV response to rapid-onset exercise and relayed the results to Integrated Human Function Core.
 5. Conducted in-house tilt study to investigate difference in hemodynamic response between tilt and stand tests.
 6. Implemented optimization algorithm (subset selection algorithm) to permit cardiovascular parameter estimation from transient hemodynamic response to gravitational stress.
 7. To increase computational efficiency and gain insight into the model structure, we developed methods that allow for systematic model reduction and simplification while preserving the dynamics of interest.
- *CPR Risk ID 43; Risk Rank 1; Risk Type I, CQs 11.02, 11.08:*
 1. In order to provide a research environment in advanced clinical decision support, we created an extensive, searchable database of physiologic waveforms, parameters, and clinical information from nearly 1000 ICU patients (10⁵ patient-hours)
 2. Developed computational methods for automatic hemodynamic event detection in large clinical databases

- *Achievements:*
 1. Developed sophisticated computational model capable of simulating the transient hemodynamic response to all major orthostatic stresses currently used in space life science research (see J.Appl.Physiol. 2002; 92:1236-1254).
 2. Current funding cycle thus far has produced 4 theses, 4 peer-reviewed publications, and 20 conference contributions.
 3. Quality of research documented through successful application for highly competitive graduate fellowships (Hugh Hampton Young Fellowship, Gottlieb Daimler- und Karl Benz-Stiftung, Germany) and travel grants (Cambridge Science Foundation).

Research Plans

- *CPR Risk ID 14; Risk Rank 1; Risk Type II, CQs 5.03:*
 1. Optimize procedures and algorithms for automated model-based extraction of physiologically relevant information from multivariate data streams, particularly those relevant to countermeasure development for orthostatic intolerance. To achieve this goal, we will:
 - Continue to expand our data archive through appropriate collaborations
 - Systematically apply the parameter estimation strategy to hemodynamic data from bed-rest subjects and astronauts
 2. Develop strategies for systematic and effective model identification and reduction, i.e., for the adaptation of model complexity to the characteristics of the available physiological data.

- *CPR Risk ID 43; Risk Rank 1; Risk Type I, CQs 11.02, 11.08:*
 Incorporate and evaluate our computational modeling technology as a core component of an advanced hemodynamic patient monitoring and decision support system in the context of critical care.

Countermeasure Development Plans

Utilize the model to simulate hemodynamic effects of proposed pharmacologic or mechanical countermeasures and to predict their impact on the system-level hemodynamic response to gravitational stress.

Collaborations

- | | | |
|---------------------------|---|--------------------|
| ▪ R.J. Cohen | MIT | NSBRI bedrest data |
| ▪ G. Williams | Harvard | NSBRI bedrest data |
| ▪ J. Meck | NASA | Astronaut data |
| ▪ J. Thomas | CCF | Cardiac data |
| ▪ K. Toska | University of Oslo | Tilt and LBNP data |
| ▪ D. Delaney | BIDMC | ICU patient data |
| ▪ Philips Medical Systems | | ICU patient data |
| ▪ Eun Bo Shim | Kwangwon National University, South Korea | GUI development |

Integrated Modeling of Cardiac Mechanical and Electrical Function
Principal Investigator: Andrew D. McCulloch

Specific Aims

- **Aim 1:** To apply our existing techniques for modeling three-dimensional cardiac mechanics and action potential propagation to develop anatomically detailed three-dimensional dynamic finite element models of regional cardiac electromechanics.
- **Aim 2:** To bridge models and data on cardiac metabolism and cellular dynamics with systems models of coronary flow, central hemodynamics, and cardiovascular regulation.
- **Aim 3:** To develop tools for using available wall motion data from medical imaging in man to validate the mechanoenergetic models and identify myocardial constitutive properties.
- **Aim 4:** To apply new models of geometric and constitutive remodeling in response to chronically altered external loading conditions to develop simulations of long-term cardiac adaptation to microgravity.
- **Aim 5:** To implement the models using modular object-oriented software engineering techniques that allow the models to be readily integrated with others through standard broker architectures for software interoperability.
- **Aim 6:** To collaborate with other prospective projects in the Integrated Human Function Core.

Accomplishments/Findings

- New cellular systems models of ventricular myocyte excitation-contraction coupling, mechanoenergetics and neurohormonal regulation, specifically: (1) a new ionic model that couples ionic currents, calcium cycling with magnesium, ATP and ADP fluxes and regulation by Mg.ATP of cellular ion pumps and exchangers (Michailova and McCulloch, 2001); (2) a new model of myocyte mechanics that combines length-dependent thin filament activation, cooperativity and cross-bridge cycling (Usyk and McCulloch, 2003); (3) a new systems model of β -adrenergic signal transduction coupled to excitation-contraction coupling mechanisms in ventricular myocytes (Saucerman *et al.*, (submitted)).
- New anatomically detailed three-dimensional models of coupled ventricular electromechanics during normal and ectopic beats (Usyk *et al.*, 2002; Usyk and McCulloch, in press).
- A new model of ventricular impulse propagation that includes the effects of regionally heterogeneous ventricular cell types (endo, epi and M-cells) on T-wave morphology (Belik *et al.*, 2003 abstract)
- New results demonstrating a slowing of ventricular impulse conduction associated with increased passive mechanical loading (mechanoelectric feedback) in experimental animals (Sung *et al.*, 2003).
- A new release of the *Continuity* software for integrative modeling that incorporates a graphical user interface, new visualization engine, client-server architecture, high-level scripting language, and support for parallel computing on Linux clusters
- A prototype of a new visual programming software environment for cell systems modeling that will become part of *Continuity*.

- New tools for building models from human tomographic images and their use for assessment of regional ventricular function *in vivo*, and for validation of three-dimensional models (Young *et al.*, 2001)

Research Plans

- To extend our signaling models to include the mechanisms of cell hypertrophy and atrophy associated with chronically altered mechanical loading in microgravity
- To develop a structurally and functionally integrated fully three-dimensional anatomically realistic and biophysically detailed model of cardiac electromechanics during exercise conditions.
- To extend the models to include regional variations in cell type and mechanical properties.
- To investigate how mechanoelectric feedback slows conduction and alters restitution dynamics.

Countermeasure Development Plans

- None

Collaborations

- Don Bers and Jose Puglisi, Loyola of Chicago
- Larry Brunton, UCSD
- Michel Sanner, The Scripps Research Institute
- Andy Feldman and James Coolahan, JHU, JPL

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- Young AA, Dokos S, Powell KA, Sturm B, McCulloch AD, Starling RC, McCarthy PM , White RD (2001) Regional heterogeneity of function in nonischemic dilated cardiomyopathy. *Cardiovasc Res* **49**(2): 308-318.

Mechanisms of Orthostatic Intolerance After Spaceflight
Janice Meck, P.I.

Specific Aims:

- Study alterations in endothelium-dependent and endothelium-independent responses in arteries and veins after spaceflight.

Accomplishments:

- Funding just initiated this past quarter
- Pitched to the STS-117 crew.

Research Plans:

- Start study

Countermeasure Development Plans

- Study outcome may lead to development of countermeasures based on endothelium-dependent or endothelium-independent control of vascular tone

Collaborations

- Drs. Michael Ziegler and Paul Mills at University of California, San Diego
- Dr. Dominick D'Aunno and Wendy Waters at Baylor College of Medicine

Project Title: “A soluble guanylyl cyclase knock-out model”

Principal Investigator: Ferid Murad M.D., Ph.D.

Specific aims:

Specific Aim 1:

To gain an understanding of the sGC-cGMP regulatory pathway in the cardiovascular system by developing an animal model with myocardium-specific and vascular smooth muscle-specific disruption of sGC gene expression. Hemodynamic consequences that result in the elimination of sGC activity in knockout mice versus wild type mice will be assessed.

Aim 1a. To produce *Lox*-targeted cardiac-specific β_1 -sGC knockout mice utilizing standard recombinant cloning and transgenic techniques. Mice will be mated with cardiac specific *Cre* mice (available through Michael Schneider at Baylor College of Medicine, Houston, TX) to obtain the double *Cre/Lox* cardiac-specific β_1 -sGC transgenic progeny and smooth muscle specific *Cre* mice (available through Franz Hofmann at Institut für Pharmakologie und Toxikologie, Munich, Germany) to obtain the double *Cre/Lox* smooth muscle specific β_1 -sGC transgenic progeny.

Aim 1b. To characterize the effects of myocardial-specific and vascular smooth muscle-specific sGC gene disruption on general appearance, ECG, arterial blood pressure, heart rate, cardiac Doppler measurements, embryonic development and histology of the heart in knockout mice versus wild type mice.

Specific Aim 2:

To determine the role of the sGC pathway deficiency on the development of orthostatic intolerance that occurs during re-adaptation to gravity, using the established tail-suspended rodent model to simulate the microgravity conditions.

Aim 2a. To determine the time course of changes in cardiovascular function induced by re-adaptation to gravity in knockout mice (myocardium-specific and vascular smooth muscle – specific) compared with wild type mice. The effects of gene disruption on ECG, arterial blood pressure, heart rate and cardiac Doppler measurements will be recorded immediately and once a day for 3 days following de-suspension in knockout mice versus wild type mice.

Aim 2b. To study the cardiovascular responsiveness in knockout mice versus wild-type mice following de-suspension. Animals will be challenged with vaso- and cardio-active agents. Pharmacological drugs will be administered immediately following de-suspension and once a day for 3 days, as cardiovascular parameters are monitored.

Accomplishments:

1. We used *Lox*- β_1 sGC targeted vector generated previous year to produce gene-targeted mouse ES line. Presently, we on the stage of blastocysts injections with gene-targeted ES clone.

2. In order to gain an advanced knowledge about regulation of sGC expression in human body we continued characterization of β_1 human sGC gene promoter region initiated previous

year and identified CCAAT binding factor (CBF) as critically important factor in β_1 sGC expression. The resulting research accomplishments were published in PNAS paper: Sharina IG, Martin E, Thomas A, Davis KL, Murad F."CCAAT binding factor regulates expression of the β_1 subunit of soluble guanylyl cyclase gene in the BE2 human neuroblastoma cell line." In press, Proc Natl Acad Sci U S A.

3. We investigated the role of the heme moiety in the basal state of human sGC and generated the constitutively active heme-deficient sGC enzyme which could be a useful reagent for a gene transfer therapy, amelioration of chronic hypertensive conditions and screening for novel inhibitors and activators of sGC. The resulting research accomplishments were published in PNAS paper: Martin E, Sharina I, Kots A, Murad F."A constitutively activated mutant of human soluble guanylyl cyclase (sGC): Implication for the mechanism of sGC activation." Proc Natl Acad Sci U S A. 2003 Aug 5;100(16):9208-13

Research plans:

To understand the sGC-cGMP regulatory pathway in cardiovascular function we will obtain an animal model with myocardium-specific and smooth muscle-specific disruption of sGC gene expression. In order to achieve this goal: a. β_1 sGC lox knockout mice will be generated utilizing the β_1 murine sGC gene Lox- β_1 sGC targeted vector created in our laboratory; b. β_1 sGC-lox mice will be bred with α MyHC-Cre mice containing a myocardium specific Cre-recombinase, to generate myocardium specific β_1 -sGC-Cre/lox knockout mice and [SM-CreER(T2)(ki)] containing smooth muscle-specific Cre recombinase to generate smooth muscle-specific β_1 -sGC-Cre/lox knockout mice.

Counter Measure Development Plans:

The generation of the sGC mouse knock-out model would help to overcome several problems associated with the study of sGC-mediated physiology. Distinctions between cGMP-dependent and -independent actions of NO, and between the physiological contributions of cGMP produced by the particulate or soluble forms of enzyme, are difficult due to the lack of selective sGC inhibitors. Furthermore, there are at least two sGC isoforms demonstrating very similar pharmacological and functional properties but markedly different expression profiles. Gene-targeted animal models, presently unavailable for sGC, could help to overcome most of these difficulties, allowing discrimination of effects on the level of a single gene. An improved understanding of the mechanisms involved will aid development of new physical and pharmacological measures to counter possible negative effects of changing environmental conditions on the human cardiovascular system. Comparisons between knockout mice and wild type mice under simulated weightlessness conditions will help to identify sGC-dependent pathways necessary for adaptation to microgravity and re-adaptation to gravity. An understanding of the biological and physiological mechanisms of sGC regulation could aid in the development of countermeasures for prevention and/or treatment of negative effects associated with adaptation to microgravity.

Effect of Simulated microgravity on the vestibulosympathetic reflex in humans

Principal Investigator: Chester A. Ray, Ph.D.

Specific Aims

- To determine muscle sympathetic nerve activity (MSNA) responses to head-down rotation before and after 1 and 7 days of head-down tilt bed rest
- To determine MSNA responses to head-down rotation during lower-body negative pressure before and after head-down tilt bed rest

Accomplishments/Findings

- Effect of aging on the vestibulosympathetic reflex. We have shown an attenuated vestibulosympathetic reflex in older subjects during rest and during an orthostatic challenge. We believe these responses are comparable to that observed during space flight.
- Melatonin attenuates sympathetic nerve responses to orthostatic stress in humans. This study indicates that high concentration of melatonin can attenuate reflex sympathetic increases to orthostatic stress in humans. These alterations appear to be mediated by melatonin-induced changes to the baroreflexes. Melatonin may be detrimental to astronauts during space flight.
- New Funding: NIDCD R01-DC6454 Vestibulosympathetic reflexes in humans
 - Please see below publications for further findings:
 1. Monahan, K. D., M. K. Sharpe, D. G. Drury, A. C. Ertl, and C. A. RAY. Influence of vestibular activation on respiration in humans. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 282:R689-R694, 2002.
 2. RAY, C. A., and K. D. Monahan. Effect of aging on the vestibulosympathetic reflex in humans. *Circulation* 105:956-961, 2002.
 3. Khan, M. H., A. R. Kunselman, U. A. Leuenberger, W. R. Davidson, Jr., C. A. RAY, K. S. Gray, C. S. Hogeman, and L. I. Sinoway. Attenuated sympathetic nerve responses after 24-hours of bed rest. *American Journal of Physiology – Heart and Circulatory Physiology* 282:H2210-H2215, 2002.
 4. Monahan, K. D., and C. A. RAY. Interactive effect of hypoxia and otolith organ engagement on cardiovascular regulation in humans. *Journal of Applied Physiology*, 93:576-580, 2002.
 5. Carter, J. R., C. A. RAY, and W. H. Cooke. Vestibulosympathetic reflex during mental stress. *Journal of Applied Physiology* 93:1260-1264, 2002.
 6. Monahan, K. D., and C. A. RAY. The vestibulosympathetic reflex during orthostatic challenge in aging humans. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 283:R1027-R1032, 2002.
 7. Carter, J. R., C. L. Sauder, and C. A. RAY. The effect of morphine on sympathetic nerve activity in humans. *Journal of Applied Physiology* 93:1764-1769, 2002.
 8. RAY, C. A., and J. R. Carter. The vestibulosympathetic reflex in humans. *Acta Physiologica Scandinavica* 177:313-319, 2003.
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 10. Carter, J. R., C. A. RAY, E. M. Downs, and W. H. Cooke. Strength training reduces arterial blood pressure but not sympathetic neural activity. *Journal of Applied Physiology* 94:2212-2216, 2003.

11. Momen A, U. A. Leuenberger, C. A. RAY, S. Cha, L. I. Sinoway. Renal vascular responses to static handgrip: The role of the muscle mechanoreflex. *American Journal of Physiology – Heart and Circulatory Physiology*, in press.
12. RAY, C. A. Melatonin attenuates sympathetic nerve responses to orthostatic stress in humans. *Journal of Physiology (London)*, in press.
13. RAY, C. A., and K. D. Monahan. Effect of endogenous opioid receptor agonist and antagonist on the vestibulosympathetic reflex in humans. *Journal of Applied Physiology*, in revision.
14. Levine, B. D., J. A. Pawelczyk, J. H. Zuckerman, R. Zhang, Q. Fu, K. Iwasaki, C. A. Ray, and C. G. Blomqvist. Neural Control of the Cardiovascular System in Space. In: *The Neurolab Spacelab Mission: Neuroscience Research in Space: Results from the STS-90, Neurolab Spacelab Mission*. (J. C. Buckey and J. L. Homick, Eds). Lyndon B. Johnson Space Center, Houston, TX. pp. 175-185, 2003.

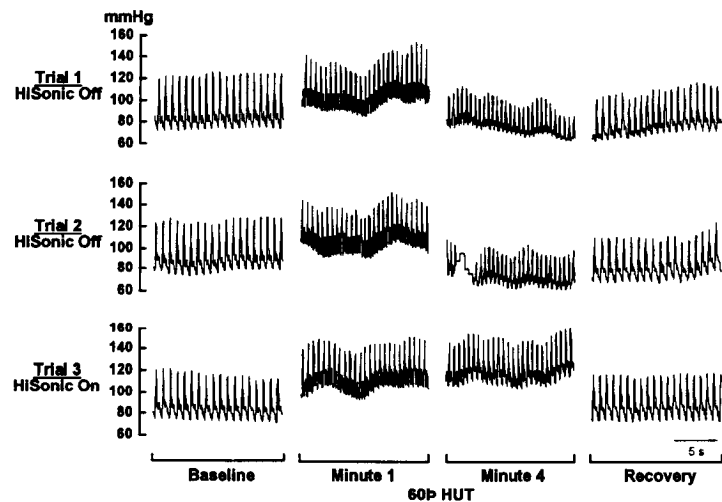
Research Plans

- To proceed with studies to determine if the vestibulosympathetic reflex can improve orthostatic intolerance after bed rest

Countermeasure Development Plans

- A possible countermeasure has been identified during this funding period and is the focused on a recently submitted grant to NSBRI. Ultrasonic stimulation of the mastoid, which is believed to stimulate the otolith organs, appears to improve orthostatic intolerance as indicated by pilot data.

Figure. Blood pressure tracings in one young woman with a history of orthostatic intolerance during three trials of head-up tilt (HUT) at 60°. During the first two trials, the women became hypotensive (pre-syncopal) by the fourth min of tilt. However, when the ultrasonic stimulation was applied to the mastoid of the women (HiSonic® device), blood pressure remained stable. These findings suggest ultrasonic bone stimulation of the mastoid can improve orthostatic tolerance.



Collaborations

- A collaboration with Michael Delp, another PI of an NSBRI Cardiovascular project, was established to determine the effects of microgravity and hindlimb unloading on cardiac mass in rats. This project was funded, in part, by both of the current NSBRI grants to Dr. Delp and myself, and the results have been published.

Ray, C.A., M. Vasques, T.A. Miller, M.K. Wilkerson, and M.D. Delp. Effects of short-term microgravity and long-term hindlimb unloading on rat cardiac mass and function. *J. Appl. Physiol.* 91: 1207-1213, 2001.

Mechanisms of Cardiovascular Deconditioning *Artin Shoukas and Dan Berkowitz*

Specific Aims

1. To determine mechanisms of impaired stroke volume response (SV) in a rat model of micro-gravity.
2. To determine molecular mechanisms of vascular (systemic and pulmonary arterial, and venous) hypo responsiveness in a rat model of micro-gravity.
3. To test pharmacologic countermeasures based on mechanisms that impair both SV responses, and vascular hypo-responsiveness in a rat model of micro-gravity.

Accomplishments/Findings

1. We have demonstrated impaired CO responses to an orthostatic challenge in rats following HLU which recovers in ~60hrs.
2. We have shown impaired alpha1-AR and non-alpha mediated contractile responses in aorta of HLU animals.
3. We have demonstrated primarily impaired alpha-1 AR contractile responses in the femoral arteries of HLU rats and demonstrated that the vascular phenomenon observed is reversible.
4. We have observed alpha-1AR specific abnormalities in mesenteric micro-vessel responses. We have demonstrated the critical role for the alpha-1b AR subtype in mediating contractile responses necessary during orthostatic stress in mesenteric vessels.
5. We have observed a decrease in alpha-1AR specific radio-ligand binding in aortic vessels from HLU animals.
6. In total body perfusion experiments we have shown that there is an increased venous capacitance and venous contractile hypo-responsiveness as one potential cause for impaired SV responses.
7. In isolated venous vessel experiments we have been able to show that there is an increase venous capacitance, as indicated by their pressure diameter relationships and the contractile properties of these vessels is diminished by either electrical stimulation or with nor-epinephrine.
8. We have been able to show that there is a drastic reduction in cardiac reserve in HLU animals and that the ability of the baroreceptor reflex to increase contractility is nearly totally abolished.
9. We have been able to show an impaired contractile responsiveness which appears to be both endothelial independent and dependent.
10. We have shown there is a significant relaxation component contributing to venous tone in mesenteric veins, which could be inhibited as a countermeasure to orthostatic intolerance.
11. In pulmonary vessels we have demonstrated:
 - Enhanced responses to endothelial cell dependent vasodilator stimuli (Ach).
 - Decreased responses to endothelial cell dependent vasoconstrictor stimuli (PE, U4).
 - Up-regulation of endothelial cell eNOS and sGC expression.
 - Enhanced vasodilator responses to endothelial cell independent vasodilator stimuli (SNP).

Flight Testing of Rats on STS-107, Columbia:

As a result of the NSBRI Team approach to counter measure development, Dr. Shoukas' entire group was asked by Dr. Michael Delp's group, with permission from NASA, to participate in flight studies of rats that were being flown on STS-107, The Columbia. Dr. Shoukas group was at Kennedy Space Center from January 13, 2003 through February 14, 2003 setting up the laboratory and performing practice experiments during the flight of STS-107 in anticipation of actual experiments that were to commence on February 1, 2003. The questions posed for the flight rats for the Columbia mission STS-107 were:

- Are the physiological changes seen in the ground based HLU rat model equivalent to the changes after long-term exposure to actual micro-gravity?
- Is the rat model of micro gravity equivalent to the astronauts exposed to long term micro-gravity?

Research Plans

These accomplishments have allowed us to refine mechanisms, begin to test countermeasures, and bridge the gap between animal models and human subjects in our understanding of micro gravity induced orthostatic intolerance.

Countermeasure Development Plans

We have finalized an external non-invasive mechanical prototype device that peristaltically pumps blood from lower extremities and abdomen towards the heart to maintain stroke volume and cardiac output during an orthostatic challenge. Provisional Patent Number: **60/440,314**. Note: A NIH-DOD grant has been submitted on May 23, 2003

entitled "Peristaltic Non-Invasive Blood Flow Assist Device". This proposal was in response to a RFA-HL-03-015, Hypo-Volemic Circulatory Collapse: Mechanics and Opportunities to Improve Resuscitation Outcomes. Funding for this proposal is for further development of the device and for human investigation and trials.

Collaborations

- **Dr. Michael Delp**
- **Dr. Janice Meck**
-

Papers:

Published or Currently In Press:

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2. Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, El-Moalem H, Page SO, Richardson CD, Winters B, Marucci L, Schwinn DA. Subtype specific regulation of human vascular α_1 -adrenergic receptors by vessel bed and age. *Circulation* 100:2336-2343, 1999.
3. Shapiro RE, Winters B, Hales M, Barnett T, Schwinn DA, Flavahan N, Berkowitz DE. Endogenous circulating sympatholytic factor in orthostatic intolerance. *Hypertension* 36:553-560, 2000.
4. Winters B, Mo X, Brooks-Asplund E, Kim AY, Shoukas A, Nyhan D, Berkowitz DE. Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in leptin deficient obese (*lep/ob*) mice. *J Appl Physiol* 89:2382-2390, 2000.
5. Varghese PG, Ricker KM, Georgakopoulos D, Berkowitz DE, Hare JM. β_3 -adrenoceptor deficiency blocks nitric oxide-dependent inhibition of myocardial contractility. *J Clin Invest* 106:697-703, 2000.
6. Dunbar SL, Berkowitz DE, Brooks-Asplund EM, and Shoukas A. "Effects of HLU on rat small mesenteric vein capacitance." *J. Appl. Physiol.* 89: 2073-2077. (2000)
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8. Brooks-Asplund EM, Berkowitz DE, Kim SY, Shoukas AA. Estrogen has opposing effects on vascular reactivity in obese, insulin-resistant male Zucker rats. *J Appl Physiol* 92:2035-2044, 2002.
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11. Ahuja A., N. D., et al. "Vasopressin Mediated Potentiation of Adrenergic Vasoconstriction in Rat Mesentery." *Anesth Analg* 96(CSA 125-) (2003).
12. Kahn S, Skaf MW, Harrison RW, Lee K, Minhas KM, Kumar A, Fradley M, Shoukas AA, Berkowitz DE, Hare JM. Nitric oxide regulation of myocardial contractility and calcium cycling: Independent impact of neuronal and endothelial nitric oxide synthase. *Circ Res.* 92: 1322-1329, 2003.
13. Berkowitz DE, White R, Li D, Minhas K, Cernetich A, Kim S, Burke S, Shoukas AA, Nyhan D, Champion HC, Hare JM. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation.* In Press.
14. Barouch LA, Berkowitz DE, Harrison RW, O'Donnell CP, Hare JM. Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation.* In Press.

Currently In Review or Being Revised:

15. Brooks-Asplund, E., Berkowitz DE, Dunbar, S. and A. Shoukas. Hindlimb unweighting attenuates cardiac output and stroke volume responses to upright tilt in male rodents. *American Journal of Physiology* (Under Revision)
16. Jung, A.S., Berkowitz, D., Brooks-Asplund, E.M., Dunbar, S. and A. Shoukas. Contribution of Carotid and Aortic Baroreceptors to Heart Rate and Pressure Changes in Acute Mice. *American Journal of Physiology* (Under Revision)
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18. Brooks-Asplund, E., Shoukas A. Baroreceptor contribution to the cardiovascular reflex responses of phenylephrine and sodium nitroprusside in the conscious rat. *American Journal of Physiology* (In Review)
19. Skaf MW, Harrison RW, Khan S, Lee K, Shoukas AA, Berkowitz DE, Hare JM. Independent impact of neuronal and endothelial nitric oxide synthases on cardiac calcium cycling. *J Clin Invest.* In review.

20. Tamhidi L., Hormel J., Berkowitz, D., Nyhan D. and Shoukas A.A. Carotid Sinus Control of Systemic Vascular Bed Capacitance in Simulated Microgravity. Am.J.Physiol. In review

In Preparation:

21. Champion HC, Bivalacqua TJ, et al. (2003). "Arginase inhibits cardiac nitric oxide bioactivity in the aging rat heart." In Preparation.
22. White AR, Shoukas AA,, Atwater S, Abbott, B and DE Berkowitz. Early Changes in Vasoreactivity in a Terrestrial Model of Microgravity are Due to an Up-regulation of the Endothelium-Dependent Nitric Oxide/cGMP Pathway. In Preparation.

Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity

*Principal Investigator: James D. Thomas, MD
Department of Cardiovascular Medicine, The Cleveland Clinic Foundation*

Specific Aims

- Assess LV mass regression during chronic volume and pressure unloading
- Validate new echo modalities to assess myocardial function in microgravity
- Validate exercise contractile reserve for detection of early LV dysfunction
- Perform core echo measurements for other NASA and NSBRI researchers
- Extend quantitative tools to 3D echo

Accomplishments/Findings

Aim #1: Impact Of Volume / Pressure Unloading On LV Properties

- 16 aortic stenosis patients and 8 with aortic insufficiency have been recruited for 3D echo
- Demonstrated a fall in LV mass from $181 \pm 42 \text{ g/m}^2$ at baseline to $149 \pm 34 \text{ g/m}^2$ at 6-weeks post valve replacement ($p=0.03$) with further fall at 6 months (limited follow-up to date).
- In 16 patients with hypertrophic cardiomyopathy, LV mass fell from $250 \pm 80 \text{ g/m}^2$ at baseline to $210 \pm 62 \text{ g/m}^2$ at 6-months post septal myectomy or ablation ($p<0.001$).

Aim #2: Non-invasive Echo Assessment Of LV Function

- We have validated echocardiographic myocardial systolic strain as a noninvasive surrogate for end-systolic elastance with diastolic strain correlating with end-diastolic pressure [1] and demonstrated regional variance in strain to be a powerful measure of success in biventricular pacing [2].
- Age-stratified reference values have been obtained in 102 normals for tissue velocity, displacement, strain rate and strain for 4 LV walls at 3 levels (base,mid,apex) [3], critical for assessing changes in microgravity.
- We have assessed the preload and inotropic dependency of tissue velocity in dogs [4] and normal humans undergoing microgravity-mimicking bedrest [5].
- Validated measurement of intraventricular pressure gradients (IVPG) via application of the Euler equation to color M-mode (CMM) Doppler transmitral flow data. [6]
- Used technique to quantify changes in diastolic suction following septal ablation in hypertrophic cardiomyopathy, a presentation that won the young investigator competition for the ACC.[7]
- We have shown CMM data to be largely independent of preload [5, 8], making it an attractive index to assess cardiovascular countermeasures in space.
- Evaluation of new 2D strain algorithm underway. Correlation with standard Doppler technique in 80 segments was 0.79. Average strain difference compared with MRI tagging approach for strain quantification is $0.7 \pm 3.7 \%$.

Aim #3: Exercise Assessment For Early Detection Of Myocardial Dysfunction During Space Flight

- 31 patients with heart failure and 15 normals underwent metabolic stress testing. Although resting IVPG by CMM was only weakly associated with VO_2max , the increment in IVPG (2.6 ± 0.8 in normals versus 1.1 ± 0.8 mmHg in patients, $P<0.05$) was most predictive of exercise capacity ($r=0.8$, $P<0.001$) [9].
- Demonstrated a simplified index of cardiac power as a way to monitor cardiac reserve in space flight [10].
- Low-grade bicycle exercise protocol has been initiated to examine new 2D strain algorithm.

Aim #4: Software for Quantitative Assessment of LV Function

- Developed stand-alone software for quantification of IVPG from CMM data and strain from TDE data
- These can be applied to DICOM data from any echo machine, such as the HDI-5000 aboard the ISS.

Aim #5: Establish An Echocardiographic Core Facility For NSBRI And NASA

- We have performed core lab analyses for NSBRI-funded bedrest studies in Boston (Richard Cohen, P.I.) and NASA funded work in Dallas (Ben Levine, P.I.).

- The latter work has demonstrated that tissue velocity shows significant preload dependency, while CMM flow propagation is virtually independent of loading conditions.
- By using these indices together, we may be able to tease out changes in cardiac performance in microgravity due to altered preload (TDI) and primary myocardial changes (CMM).
- Aging Decreases Intraventricular Pressure Gradient Response to Preload Changes (Popovic AHA abstract)

Research Plans

- INCREASE RECRUITMENT OF PATIENTS AND DATA ANALYSIS (AIM #1)
- UTILIZE 2D STRAIN TECHNIQUE ALONG WITH TDE STRAIN AND CMM IVPG IN ASSESSMENT OF MYOCARDIAL PERFORMANCE (AIMS #2 AND #4)
- CORE LAB DATA ANALYSIS (AIM #5)

Countermeasure Development Plans

- Assessment of midodrine for orthostatic intolerance
- Assessment of exercise for prevention of atrophy

Collaborations

- Core analyses for the NSBRI funded bedrest studies in Boston (Richard Cohen: PI)
- Core analyses for NASA funded work in Dallas (Ben Levine, PI).
- We are also collaborating with Dr. Lakshmi Putcha (Johnson's Space Center), to quantify hepatic flow.
- We are also collaborating with Drs. Lorell and Schneider concerning the measurement of cardiac strain in rodent models of microgravity.
- Through the Smart Medical Care Team, we are also working with Dr. Lawrence Crum to couple improved ultrasound diagnostics with his high intensity focused ultrasound (HIFU) method for cauterization and tumor ablation.

Publications

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RENAL AND CARDIO-ENDOCRINE RESPONSES IN HUMANS TO SIMULATED MICROGRAVITY

Principal Investigator: Gordon H. Williams, M.D.

Specific Aims

- To investigate the influence of age and gender on the pattern of renal sodium handling and the acute responsiveness of the RAAS following simulated microgravity exposure.
- To investigate the effects of simulated microgravity on myocardial electrical stability (joint project with Dr. Richard Cohen from MIT).
- To investigate the effects of the alpha-1 agonist midodrine, as a countermeasure against orthostatic intolerance following simulated microgravity exposure in men and women.

Accomplishments/Findings

- During the past several years, a sixteen day-head-down tilt bed rest protocol was used to investigate the specific aims mentioned above. We have found that simulated microgravity leads to:
 - Initial loss of urinary volume and sodium followed by reestablishment of sodium balance through activation of the renin-angiotensin system [14; 3.05];
 - Continuous potassium loss [14; 3.05];
 - A dissociation between renin and aldosterone responses to simulated microgravity and up-right tilt [14; 3.05];
 - Cardiac electrical instability [13; 3.01].
 - Also, subjects intolerant to head-up tilt pre-bed rest are intolerant post-bed rest, but the reverse is not true, suggesting individual susceptibility to orthostatic intolerance independent of simulated microgravity [14; 3.08].
 - Finally, in a randomized double-blinded trial, we observed that midodrine was an efficient countermeasure for the treatment of orthostatic intolerance following simulated microgravity [14; 3.09].
- Thirty-two males and 10 females have been studied (four females left to complete). Preliminary data in women suggests a much poorer tolerance to orthostatic stress at baseline and after bed rest, as well as a similar activation of the renin-angiotensin system and dissociation between renin and aldosterone responses to simulated microgravity.
- **Major accomplishments:**
 - Ramsdell CD, Mullen TJ, Sundby GH, Rostoft S, Sheynberg N, Aljuri N, Maa M, Mukkamala R, Sherman D, Toska K, Yelle J, Bloomfield D, Williams GH, Cohen RJ. *Midodrine Prevents Orthostatic Intolerance Associated with Simulated Spaceflight*. *Journal of Applied Physiology*, 90: 2245-2248, 2001.
 - Grenon SM, Sheynberg N, Hurwitz S, Xiao X, Ramsdell CD, Ehrman MD, Mai CL, Rostoft Kristjansson S, Sunby GH, Cohen RJ, Williams GH. *Renal, Cardio-Endocrine, and Cardiovascular Responses to Simulated Microgravity in Male Subjects on a High-Salt Diet* (Submitted to *Journal of Investigative Medicine*)

- Hurwitz S., Williams GH. *Diurnal variation of aldosterone and plasma renin activity: Timing relation to melatonin and cortisol and consistency after prolonged bedrest.* (Submitted to Journal of Applied Physiology)
- Xiao X, Mukkamala R, Sheynberg N, Grenon SM, Ehrman MD, Mullen TJ, Ramsdell CD, Williams GH, Cohen RJ. *Effects of Simulated Microgravity on Closed-loop Cardiovascular Regulation and Orthostatic Intolerance: Analysis by Means of System Identification.* (Submitted to Journal of Applied Physiology).

Research Plans

- To investigate the use of pre-bed rest tilt-test as a means to screen countermeasures against orthostatic intolerance and assess the influence of these countermeasures on the renal and endocrine responses to orthostatic stress.
- To test the hypothesis that the countermeasure found to be most effective in during the pre-bed rest tilt-test will increase orthostatic tolerance post-simulated microgravity and do so by modifying the renal and endocrine responses to orthostatic stress.
- To investigate, in older men, the effects of different countermeasures in reducing cardiac electrical instability, and to study the effects of these countermeasures on baseline orthostatic tolerance and hormonally mediated volume regulatory systems.
- To test the hypothesis that the countermeasure found to be most effective in screening test will decrease cardiac electrical instability after simulated microgravity, and do so by modifying the renal and endocrine responses to simulated microgravity.

Countermeasure Development Plans

- Orthostatic intolerance: Midodrine (10 mg), Single Bout of Maximal Exercise, and Salt and Water Loading will be studied in a randomized, double-blinded placebo-controlled trial.
- Changes in Cardiac Electrical Stability: Eplerenone (25 mg), Metoprolol (25 mg), and Potassium and Magnesium Replacement will be studied in a randomized, double-blinded placebo-controlled trial.

Collaborations

- Dr. Williams (Harvard Medical School) has enjoyed a successful collaboration with Dr. Richard Cohen (MIT) during the preceding six years in conducting bed rest studies, and both have developed a close working relationship. Several of the studies done at Harvard and MIT are complementary to one another
- Dr. Williams has also enjoyed a close working relationship with Dr. Jim Thomas (Cleveland Clinic Foundation) related to cardiac echography.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL PROGRAM REPORT November 5, 2003

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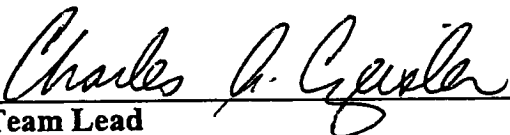
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I. ABSTRACT

The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During long-duration space flight, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under space flight conditions. This team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles, altered or reduced sleep/rest opportunities, high levels of automation, and habitation in a remote, inaccessible location. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during long-duration space flight.

The goals of the Human Performance Factors, Sleep and Chronobiology (HPFSC) Team are to: (1) Characterize and quantify the adverse effects of long-duration space flight on sleep and circadian rhythms; (2) Characterize and quantify the effect of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance; (3) Understand the basic mechanisms underlying the deterioration of sleep, circadian organization and human neurobehavioral function during space flight; (4) Develop high-fidelity mathematical models of performance based on circadian organization and sleep-wake history; (5) Develop effective countermeasures to optimize sleep and facilitate circadian adaptation in the space environment and thereby maintain optimal neurobehavioral performance; (6) Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity, light exposure and neurobehavioral performance during space flight, with possible spin-off applications on Earth.

The team research objectives are driven by the Critical Path Roadmap (2000) related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves ten ground-based research projects. Many of the projects impinge on more than one critical risk within CPR Risk Area 19 (Critical Questions #s 6.05, 6.06, 6.07, 6.08, 6.18, 6.21) and CPR Risk Area 20 (Critical Questions #s 6.11, 6.12, 6.15) The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organismic, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems. The resultant research and team interactions are intended to accelerate progress on countermeasures that reduce the risk of human neurobehavioral or physiological performance failure during space exploration. Specific countermeasures under study include lighting, napping, scheduling and non-photic interventions such as meal-timing, exercise, and melatonin administration.

II. INTRODUCTION

The need for sleep and an entrained circadian pacemaker have a sustained influence over many biomedical systems essential for maintaining astronaut physical condition, mental health, and performance capability. Dysfunction of sleep and circadian systems can adversely affect an organism's ability to respond to environmental challenges and has been linked to physiological and psychological disorders. The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During exploration class space missions, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under such conditions. It has been demonstrated that both acute gravitational changes and space flight disrupt circadian rhythms and reduce sleep. Since circadian disruption and sleep loss result in both physiological and performance deficits, this team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles and altered or reduced sleep/rest opportunities that may involve extended durations of wakefulness. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during exploration class missions. This area has a high degree of relevance to a number of cardiovascular and immune changes, neurovestibular alterations and nutritional needs, and behavioral and psychological health in space flight.

The Human Performance Factors, Sleep and Chronobiology Team's program addresses risks and hazards in space flight that have been identified in the Human Behavior and Performance Discipline Area of the Critical Path Roadmap Baseline Document (2000). Specifically:

- Human Performance Failure Because of Sleep and Circadian Rhythm Problems (19)
- Human Performance Failure Because Of Human System Interface Problems and Ineffective Habitat, Equipment, Design, Workload, or In-flight Information and Training Systems (20)

Our team has the following three **risk-based goals** for its program:

Goal 1: *Reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase, amplitude, period, or entrainment during space exploration.*

Goal 2: *Reduce the risk of human neurobehavioral or physiological performance failure due to acute or chronic degradation of sleep quality or quantity during space exploration.*

Goal 3: *Reduce the risk of human neurobehavioral or physiological performance failure due to habitat design, equipment design or workload distribution during space exploration.*

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral and physiological performance decrements that occur during long-duration space flight. These countermeasures may include behavioral, pharmacological, environmental light or other adaptive approaches such as meal timing to maintain function and performance under the adverse conditions of exploration class space missions.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral performance decrements that occur during long-duration space flight. The team research objectives are driven by the Critical Path Roadmap related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves nine ground-based research projects. The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organism, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems.

In 2001, the HPFSC Team was substantially restructured. The current team is comprised of ten PIs, seven of whom are new NSBRI investigators. Four of these seven new NSBRI investigators are new to the space science community, a direct result of the recruitment efforts made within the science community. In order to achieve the goals listed above, the Human Performance Factors, Sleep and Chronobiology Team has identified the following six interrelated themes within this research area:

- A. **Effects of long-duration space flight on sleep and/or circadian rhythmicity.** The focus of this theme is to identify and understand the mechanism underlying the effect of long-duration space flight (microgravity, altered light intensity, loss of geophysical cues, isolation, altered physical activity, etc.) on neurobiologic, endocrinological, and behavioral functions (molecular, cellular and organismic) that control sleep and circadian systems.
- B. **Effects of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance.** The focus of this theme is to identify and to understand the mechanisms underlying the acute and chronic adverse effects that sleep loss, sleep disruption, and/or circadian dysfunction have on critical physiologic and performance parameters during long-duration space flight (e.g., neurophysiologic function, physiological alertness, vigilance, cognitive performance, mood/morale, problem solving and communication).
- C. **Predictive modeling of performance based upon circadian organization and sleep homeostasis.** This theme is concerned with the development of analytical or phenomenological mathematical models that predict individual human performance capability by involving multiple subsystems (e.g., circadian rhythmicity, sleep homeostasis, work-rest schedules, etc.) as an integrated unit across levels of organization, and by estimating the impact of countermeasure use designed to optimize human physical and/or neurobehavioral performance.
- D. **Countermeasures to optimize sleep and facilitate circadian adaptation in space and maintain optimal neurobehavioral performance.** The research program of this team will not only define the impact of the space environment on sleep and circadian rhythmicity and the effects of the sleep loss and circadian dysfunction on performance but also will develop methods to counter the adverse physiological and behavioral events. These countermeasures may include behavioral, pharmacological, environmental light or other adaptive approaches to maintain function and performance under the adverse conditions of long-duration space flight.
- E. **Monitoring and assessment during space flight.** This theme deals with the development of methods for monitoring the status of sleep, sleep homeostasis and circadian organization, as well as technologies that monitor ambient lighting conditions on space shuttle and ISS and assess and update the current functional status or performance capability of the individual

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral and physiological performance decrements that occur during long-duration space flight. The initial strategic research program for the Human Performance Factors, Sleep and Chronobiology Team involves nine research projects that collectively address the five research themes described above. The schematic of the circadian and homeostatic regulation of sleep and alertness and physiological functions shown in Diagram 1 illustrates the relationships between the ten current ground-based experiments that comprise the NSBRI Human Performance Factors, Sleep and Chronobiology Team, with the principal targets of each project indicated. This diagram illustrates the interrelated nature of these projects, designed to fill critical gaps in knowledge that need to be filled in order to develop effective countermeasures for long-duration space flight. Each of the individual projects is summarized below and in Table 1, including which goal(s) are addressed and countermeasure targets.

Brainard et al.: Optimizing Light Spectrum for Long Duration Space Flight

The physiological changes caused by disturbed circadian rhythms and altered sleep-wake patterns can result in decrements in alertness, concentration, and performance. This project addresses these risk factors, which threaten the safety of personnel and the objectives of space missions as stated in Goals 1 and 3.

Countermeasure targets include:

1. Identification of the optimum spectrum for photic entrainment and resetting of the circadian pacemaker.
2. Design specifications for space suit visors and the windows used in space vehicles and habitats;
3. Engineering parameters for the ideal spectral distribution for illumination of general living quarters during space exploration.

Czeisler et al.: Circadian Entrainment, Sleep-Wake Regulation & Performance during Space Flight

The intent of this project is to develop countermeasures to facilitate adaptation of the human circadian pacemaker to the 24.65-h day length of Mars, which is outside the range of entrainment of the human circadian pacemaker given the weak synchronizing stimuli within the Martian habitat. This project applies to Goals 1, 2 and 3.

The primary *countermeasure target* is to evaluate the efficacy of intermittent bright light pulses as a treatment to reduce the risk of the misalignment of circadian phase, sleep disruption, associated decrements in neurobehavioral performance and reduction in nocturnal growth hormone secretion experienced by individuals exposed to the 24.65h Martian day.

Dinges et al.: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

Using a response surface experimental paradigm (RSM), this project seeks to reduce neurobehavioral deficits and fatigue due to inadequate sleep in astronauts by investigating how variations in sleep duration and its circadian placement relate to the return of performance per time invested in sleep. This project applies to Goals 2 and 3.

Countermeasure targets include determination of the amount of naptime necessary to compensate for reduced nocturnal sleep periods for the prevention of cumulative sleepiness and performance deficits.

Foster et al.: Novel Photoreceptor Mechanisms Regulating Circadian Rhythms, Sleep, Body Temperature and Heart Rate.

The results from these experiments will be integrated with studies on human subjects undertaken by other team members (particularly Project 1) and allow us to understand how the classical rod and cone visual system interacts with the non-rod, non-cone ocular photoreceptors to regulate physiology and behavior. Space flight has been associated with abnormal circadian rhythms, sleep-wake patterns, mood, concentration, and alertness.

Countermeasure targets include the design of new lighting sources that are either highly effective in regulating these novel photoreceptors or leave them largely unstimulated. This project applies to Goals 1 and 2.

Fuller et al.: Primate Circadian Rhythms in the Martian Environment

This project is focused on the ability of the circadian time system to synchronize to the Martian photic environment (spectrum and period) by examining the effects of 1.0, 1.5 and 2.0G on the period of the circadian pacemaker. A G vs. period model will be developed to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. Long-term physiological and behavioral responses will be examined.

Countermeasure targets include the use of timed bright light pulses on circadian entrainment. This program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of males and females to altered lighting and gravitational environments. This project applies to Goals 1 and 3.

Jewett et al.: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

The intent of this project is to further develop and refine our mathematical dynamic stimulus processing model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting system especially those which are present in space. The mathematical Neurobehavioral Performance model validated against performance data collected will result in the development of a user-friendly Performance Simulation Software program. This project applies to Risk-based goals 1 through 3 and Non-Risk-Based Goal 4 (see below).

Countermeasure targets include the design of shift schedules to allow astronauts to receive available bright light at appropriate times for proper circadian alignment with their sleep/wake schedules.

Menaker et al.: A Model of Circadian Disruption in the Space Environment

This project proposes to evaluate the effects of “constant” conditions and shift work schedules on the maintenance of circadian rhythmicity when the central and peripheral structures are abnormally phased. The resulting abnormal circadian organization is “dysphasia.” This project applies to Goal 1.

Countermeasure targets include an evaluation of meal timing, melatonin administration, forced exercise, and short pulses of complete darkness as a treatment to reduce the risk of circadian dysphasia.

Morin et al.: Circadian and Vestibular Relationships

This project seeks to determine the route by which a correlate of the non-photoc stimulus, i.e., locomotion, might gain access to the circadian rhythm system and shift rhythm phase. It has also opened the possibility that the vestibular system is a specific route by which sensory information related to head movement might gain access to the circadian system. This project applies to Goal 1.

Countermeasure targets include an evaluation of a non-locomotor, non-photic three-dimensional motion stimulus to activate functionally the vestibular and circadian systems, laying the groundwork for the future development of novel approaches for the treatment of space motion sickness and for resetting circadian phase.

Tosini et al.: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

The goal of this project is to understand the mechanisms responsible for the desynchronization of circadian rhythm in locomotion and the enzymes responsible for the production of melatonin. Investigating the effect that internal desynchronization has on the immune response and motor and cognitive performances. This project applies to Goal 1.

Countermeasure targets include an evaluation of the use of melatonin as a pharmacological agent to counteract desynchronization of the circadian rhythms.

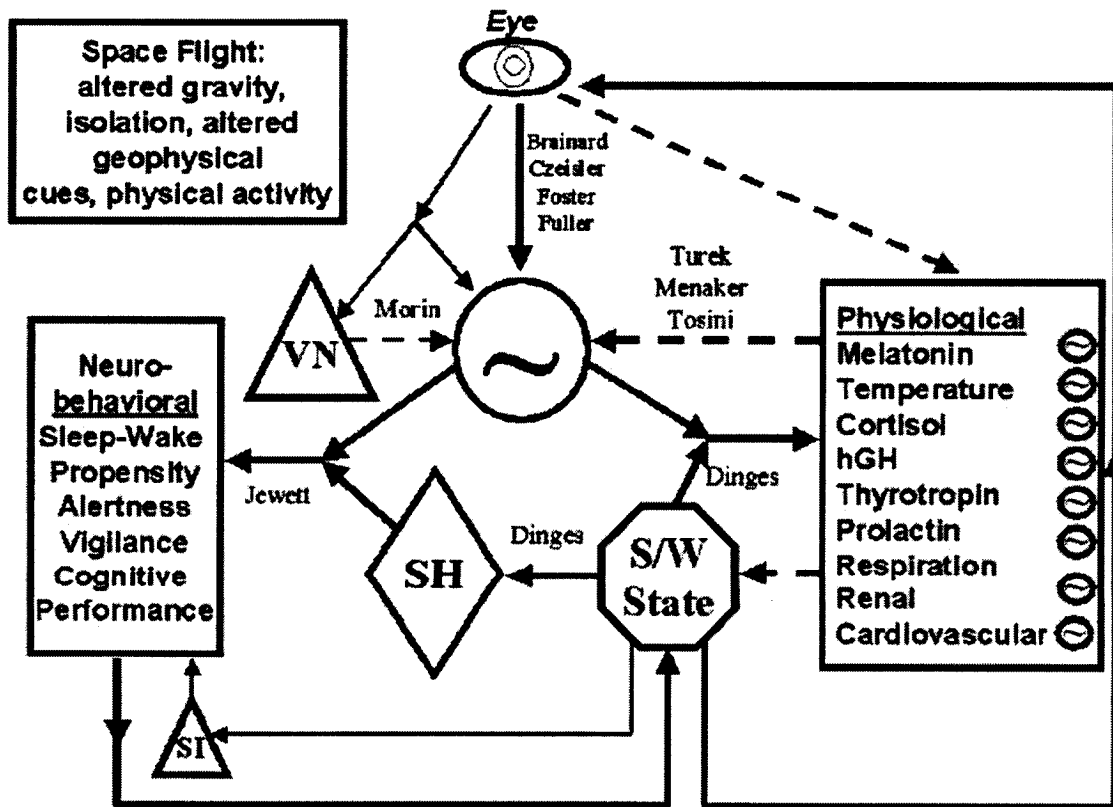
Turek et al.: Animal Model for Sleep Loss and Circadian Disruption

This project will focus on determining the effects of 12 hours of imposed wakefulness on circadian rhythms, sleep-wake cycles, neurobehavioral and motor performance measures during normal active and inactive periods. This project applies to Goals 1 and 2.

Countermeasure targets include treatment exercise and with either physiological or pharmacological dose of melatonin reduce the effects of circadian disruption and sleep loss as well as alleviate the adverse effects associated with work at different times of day.

Diagram 1. Description of Current (2003) Program for Human Performance Factors, Sleep and Chronobiology

This diagram illustrates the relationships between the different physiological systems investigated by the different projects on the team. Illustrated is the influence of the retinal light exposure on the human circadian clock (circle with the oscillator symbol ~) and the influence on the sleep-wake state (S/W), and their effect on a number of physiological variables (melatonin, temperature, etc.). A combined influence of the circadian clock and sleep-wake is exerted on neurobehavioral variables (sleep-wake propensity, alertness, etc.). The sleep-wake state influence is illustrated via the intermediary of the sleep homeostat (SH), and sleep inertia (SI). The global influence of factors associated to Space Flight (micro gravity, isolation, etc.) on the sleep and circadian systems is also represented. The interaction of the Vestibular Nucleus (VN) and its output pathways with the circadian pacemaker is being investigated by one project.



HUMAN PERFORMANCE FACTORS, SLEEP AND CHRONOBIOLOGY PROGRAM

Table 1. Project Research Activities

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
BRAINARD/Optimizing Light Spectrum for Long Duration Space Flight	Goals 1, 3	Optimum light spectral distribution	Healthy male and female human subjects	Develop melatonin fluence-response curves below 440 nm and above 600 nm in human subjects. Develop action spectrum between 400 and 700 nm in subjects with undilated pupils	Identification of optimum light spectral characteristics for maintaining or adjusting circadian phase and sleep-wake cycle in astronauts and ground control workers. Preliminary test of monochromatic stimuli for phase shifting human circadian rhythms	Preliminary development and testing of prototype fluorescent lamps for pre-launch and in-flight use as lighting countermeasure. Assist in designing a novel light panel for circadian stimulation. Assist in developing protocols for comparing head mounted light therapy devices
CZEISLER/Circadian Entraining, Sleep-Wake Regulation & Performance During Space Flight	Goals 1, 2, 3	Intermittent bright light pulses	Healthy male and female human subjects scheduled to non-24-hour day lengths in an environment shielded from periodic, 24-h time cues	Quantification of the intrinsic period and the limits of entrainment of the human circadian pacemaker; investigation of the effect of circadian misalignment on sleep, neurobehavioral performance and neuroendocrine function	Preliminary evaluation of the efficacy of intermittent bright light pulses on circadian entrainment to non-24-hour work-rest schedules, as required on Mars	Full-scale clinical trial of age and gender matched astronaut surrogates living for extended durations on a non-24-hour work schedule while exposed to intermittent bright light at the most effective wavelength
DINGES/Countermeasures to Neurobehavioral Deficits from Partial Sleep Loss	Goals 2 and 3	Naps and split sleep schedules	Healthy male and female human subjects	Mathematically track neurobehavioral performance deficits associated with chronic sleep restriction. Examine sleep efficiency and architecture during restricted sleep periods at different circadian phases	Develop response surface map paradigms to further understand the interaction between sleep duration, sleep-wake placement and neurobehavioral functioning	Development of optimal sleep-wake schedules (including main and supplementary sleep episodes) to ensure maintenance of high level neurobehavioral functioning

<p>FOSTER/Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate.</p>	<p>Goals 1 and 2</p>	<p>Mechanisms and role of novel ocular photoreceptors.</p>	<p>Normal and transgenic mouse models</p>	<p>Telemetry of body temperature, heart rate, EEG. Fos expression in the VLPO of <i>rd/rd cl</i> and normal mice. Initiate Proteomics and microarray projects to identify genes and proteins associated with novel photoreception.</p>	<p>Action spectroscopy of non-rod, non-cone responses to light. Tract-tracing of non-rod, non-cone photoreceptive pathways. Primary analysis of genes and proteins associated with novel photoreception.</p>	<p>Advanced analysis of genes and proteins associated with novel photoreception including the development of transgenic mice to place candidate genes into a functional context.</p>
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Note:

- Goal 1:** Reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase, amplitude, period, or entrainment during space exploration.
- Goal 2:** Reduce the risk of human neurobehavioral or physiological performance failure due to acute or chronic degradation of sleep quality or quantity during space exploration.
- Goal 3:** Reduce the risk of human neurobehavioral or physiological performance failure due to habitat design, equipment design or workload distribution during space exploration.

<p>FULLER/Primate Circadian Rhythms in the Martian Environment</p>	<p>Goals 1 and 3</p>	<p>Bright light pulses</p>	<p>Rhesus monkeys as human surrogates. Large-diameter centrifuge to produce altered environment. Controlled lighting period, intensity and spectra. Long duration exposure in controlled animal facilities.</p>	<p>Determine the effect of altered gravity on primate circadian rhythms, principally the endogenous clock period.</p>	<p>Enhance entrainment response to low light (ISS, Martian habitat), reddish light (Mars), and non-24 hour schedules by means of exposure to light pulses. Definition of bright light source for light pulses. Studies will address timing and efficacy of bright light exposure.</p>	<p>Projected application of bright light pulses to prevent loss of circadian entrainment, sleep and rhythm disturbances, performance decrements.</p>
<p>JEWETT/Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure</p>	<p>Goals 1,2 and 3</p>	<p>Design of rest/work and sleep/wake schedules</p>	<p>Previously collected human data;</p>	<p>Determine the nature of amplitude recovery dynamics of human circadian system.</p>	<p>Design shift and sleep schedules for proper circadian alignment. Validate refined circadian amplitude dynamics of light model with data from new human phase shifting experiments</p>	<p>Incorporate refined light model into circadian components of neurobehavioral performance model and predict the performance in human phase shifting experiments</p>
<p>MENAKER/ A Model of Circadian Disruption in the Space Environment</p>	<p>Goals 1</p>	<p>Coupling between multiple circadian oscillators</p>	<p>Transgenic rat incorporating a circadian luciferase reporter gene</p>	<p>Description of system disintegration under simulated space flight conditions</p>	<p>Repair system disintegration with timed application of light, food and melatonin</p>	<p>Transfer working countermeasures to humans</p>
<p>MORIN/Circadian and Vestibular Relationships</p>	<p>Goals 1</p>	<p>Anatomical & functional issues linking the vestibular & circadian systems</p>	<p>Anatomical tract tracing using retro and anterograde transport of labels</p>	<p>Understanding the basic anatomical & functional pathways linking vestibular & circadian</p>	<p>N/A</p>	<p>N/A</p>

<p>TOSINI/Long-term Exposure to Dim Light Desynchronizes the Circadian System of Rats</p>	<p>Goal 1</p>	<p>Coupling between central and peripheral oscillators</p>	<p>Study of brain regions for stimuli responses known to alter vestibular functions Phase shifts in circadian rhythms Measuring expression of gene in peripheral tissues</p>	<p>systems</p>	<p>Use of melatonin as synchronizing agent</p>
<p>TUREK/Animal Model for Sleep Loss and Circadian Disruption</p>	<p>Goals 1 and 2</p>	<p>Exogenous melatonin administration and exercise</p>	<p>Two strains of mice exposed to 12 hours of forced activity each day for 10 consecutive days</p>	<p>Determine the effects of 10 days of chronic partial sleep loss on sleep/wake, circadian rhythms & performance</p>	<p>Testing the effectiveness of countermeasures (melatonin, exercise) using a mouse model</p>
					<p>Transfer working countermeasures to humans and provide data to refine mathematical models</p>

In addition to the focus of the Human Performance Factors, Sleep and Chronobiology Team on achieving Risk-based Goals, there are also a number of important, **non-risk based goals** that the team is targeting, as follows:

Goal 4: *Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance during space flight.*

To achieve this goal, current studies are being conducted to assess the potential of using the Actiwatch-L (a wrist-worn light and actigraphy recording device already approved for space flight) to monitor sleep and light exposure of individual crew members while in space. This device could replace more extensive polysomnography devices used in more recent studies of sleep in space. Studies are also underway that compare the wrist-level Actiwatch-L light recordings with eye-level light measurements. Work is progressing on the use of the Actiwatch-L measurements as inputs to a mathematical model that can then predict the level of sleep homeostasis, phase of circadian rhythmicity and relative neurobehavioral performance levels.

Goal 5: *Develop new methods for monitoring ambient and retinal light exposure (illuminance/photopic lux, broadband visible irradiance, and circadian effective illuminance/circadian lux) on board space shuttle and ISS during space flight and on planetary habitats.*

For measurement of retinal light exposure in space, please see Goal 4 above. For ambient light exposure, wall-mounted ambient light recording devices have been tested aboard the Space Shuttle in the Neurolab flight. The team's current studies will help determine the circadian effective illuminance and irradiance levels, and then these recording devices can be refined to measure circadian-activating light levels more precisely.

Goal 6: *Develop Earth-based applications of technologies for non-invasively monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance for industrial and medical use.*

The polysomnography device that was developed for the recording of sleep in space in the Neurolab Shuttle flight have become a useful, wire-free device for recording polysomnography in lab-based and home-based basic science and clinical studies. This technology has the advantage of being appropriate for use when ambulatory, and straight-forward enough for a trained person to apply to themselves.

The use of salivary melatonin as a marker of circadian phase has been applied in both space and on Earth and is a technology that allows the validation of experimental and modeling results in field studies in which plasma melatonin measurements would not be possible.

Mathematical models that are developed to predict neurobehavioral performance in space are also being used to determine appropriate shift scheduling, light exposure, sleep timing, and countermeasure applications for shift workers, pilots, military and medical personnel, and transportation workers who also face the challenges of restricted sleep and circadian misalignment here on Earth. Neurobehavioral test batteries that are developed for these projects are useful for the validation of mathematical models in field and laboratory studies as well.

Goal 7: *Develop Earth-based applications of high-fidelity mathematical models of performance based on circadian organization and sleep-wake history for industrial and medical use.*

The mathematical models of performance that are being developed in this project can be applied to any Earth-based situation in which it would be helpful to know the effects of a sleep/wake

schedule and a light exposure pattern on resulting neurobehavioral performance (e.g., shift workers, pilots, military and medical personnel, and transportation workers). Therefore, the mathematical models developed here have been programmed into user-friendly simulation software that can be used by anyone to predict neurobehavioral performance given light exposure levels and sleep/wake history. This software is updated with model revisions and user-interface improvements on a regular basis.

Goal 8: *Develop Earth-based applications of technologies developed to reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase amplitude, period or entrainment.*

The studies conducted here improve our understanding of the effects of light on the human circadian system, and the role that the circadian system plays in neurobehavioral performance. These findings are incorporated into our mathematical models on an ongoing basis. This allows us to then determine the best light schedule and intensities to reduce the risk of performance failure by appropriately aligning the circadian system with the work/rest schedule. This technology is already currently in use in transportation, military and industrial settings here on Earth.

Goal 9: *Develop Earth-based application so technologies developed to reduce the risk of human neurobehavioral or physiological performance failure due to acute a chronic degradation of sleep quality or quantity.*

Our projects will help determine the amount and timing of sleep that best allows people to work extended and/or misaligned shifts with the least risk of performance failure. These findings will also be incorporated into the mathematical model being developed here. The model can then be used to help schedule rest/nap/sleep times so that they are the most effective in improving performance levels.

Goal 10: *Develop Earth-based applications to technologies developed to reduce the risk of neurobehavioral or physiological performance failure due to extended duration work schedules (e.g., on-call schedules used in medical training, nuclear power plant shutdowns, military operations) or night shift work.*

Studies investigating the effects of extended duration work schedules in these projects allow us to determine the best timing of countermeasures (light exposure, naps, melatonin, etc.) to improve performance. These findings are completely applicable to any extended duration work schedules used here on Earth.

Goal 11: *Integrate research and analysis*

Our goal is to integrate research within the Human Performance Factors, Sleep and Chronobiology Team, with other teams, and with work being done by Team investigators not directly supported by NSBRI.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The program accomplishments of each of the individual projects is summarized below:

Project 1: Optimizing Light Spectrum for Long Duration Space Flight

PI: *George Brainard, Ph.D.*
Thomas Jefferson University

Research Focus: Develop and improve pre-launch and inflight lighting countermeasures by identifying optimal spectral transmission characteristics for visors and windows, and engineering the ideal spectral distribution for illumination of living quarters.

Specific aims:

Test the hypotheses that:

1. Wavelengths of light below 440 nm and above 600 nm are active in regulating melatonin secretion via measurement of fluence response curves in humans;
2. There will be a loss of sensitivity to monochromatic light when the eyes are not pharmacologically dilated during the melatonin suppression test;
3. There will be a shift in spectral sensitivity of light regulation of melatonin secretion when the eyes are not pharmacologically dilated.

Research Progress 11/1/2002-10/31/2003:

The long term goal of our research is to determine the best wavelengths of light for use as a countermeasure during long duration space flight, as well as for adjusting circadian and sleep disruption in civilians. A melatonin suppression fluence-response curve for monochromatic light at 420 nm is now completed (N=8). A key finding is that the fluence-response relationship between 420 nm exposure and melatonin suppression is univariant with wavelengths between 440 and 600 nm. This finding has practical importance to astronauts in long duration space flight since 420 nm irradiance is greatly increased outside the earth's atmosphere (e.g. Space Shuttle and International Space Station).

Progress has also been made in establishing a melatonin suppression fluence-response curve for 630 nm. This required the design and construction of more powerful equipment to reach the desired intensities for wavelengths above 600 nm. So far, over 50 individual night studies have been completed in the long wavelength portion of the spectrum. Testing these wavelengths has relevance for astronauts who have to adapt to extraterrestrial environments that have spectral characteristics different from those found on Earth. For example, there is an abundance of long wavelengths above 600 nm in Martian skylight.

A within-subjects study quantifying the role of pupil dilation on melatonin suppression has been completed. Volunteers (N=7) completed two fluence-response curves at 460 nm, one with pupils pharmacologically dilated and one with pupils freely reactive. The key finding is that there is up

to a 36% loss of sensitivity to 460 nm light for melatonin regulation when the pupils are free to respond to light stimuli. The same subjects are currently participating in the study to test the third and final aim, whether the action spectrum for pupil dilation matches the action spectrum for circadian regulation. Unless these two action spectra are identical, then a freely reacting pupil will modify the resulting circadian action spectrum. It will be important to further characterize both wavelength and intensity responses in freely constricting eyes in order to practically utilize action spectrum data in optimizing light as a countermeasure to circadian disruption during long duration space flight. It is expected that astronauts' eyes will be freely reactive during long duration space flight.

The above progress can be used to optimize the total lighting environment of astronauts on long duration space exploration missions. These data can be used to 1) improve light treatment as a countermeasure for circadian and sleep-wake disruption in space flight, 2) identify the best spectral transmission for space suit visors and the windows used in space vehicles and habitats, and 3) engineer the spectral distribution for illumination of general living quarters during space exploration.

The current project (2001-2004) will complete the concept formulation component of determining the countermeasure for sleep disturbance and circadian disruption related to the light spectrum. In the new funding cycle (2004-2007) it has been proposed that this project will progress to initial demonstration of laboratory/clinical efficacy in human subjects (Countermeasure Readiness Levels 5 and 6). Specifically, by July, 2004, our laboratory will be able to test prototypes of fluorescent lamps that are enriched in the blue portion of the spectrum. If these prototype lamps were to perform as predicted, their increased efficacy for circadian phase-shifting will make them stronger stimuli than the white fluorescent lamps currently used by NASA as a pre-launch countermeasure for circadian and sleep disruption. In addition, these prototype lamps may also be an appropriate choice for in-flight countermeasure use during Space Shuttle, International Space Station and longer duration exploration-class missions. The current progress on this project has made it possible to move directly to development and testing of this lighting countermeasure technology. Upon completion of these studies, NASA may want to consider the immediate implementation of this new technology for in-flight testing.

General lighting systems for astronauts for manned space programs and space stations are often comprised of light sources which provide wavelengths and intensities for optimal vision in space vehicles (Man-Systems Integration Standards, NASA-STD 3000, 1995). For example, the specification on Lighting Intensity Design (8.13.2.1 A) reads: "Light level or intensity should be sufficient to allow the crew members to perform their visual tasks efficiently...[for most nominal work and living space areas]." Although it is obviously useful to optimize visual stimulation of astronauts with the best intensities and wavelengths for photopic vision, those lighting characteristics are not necessarily optimal for reinforcing circadian entrainment.

Our progress to date on this project has resulted in three peer-review publications (with a fourth currently under review), four book and proceedings chapters, and fifteen abstracts which reference NSBRI support. Understanding the relative potency of different wavelengths for circadian stimulation is a critical step towards optimizing light as a specific countermeasure and a general illuminant in all long duration space exploration facilities.

Project 2: Circadian Entrainment, Sleep-Wake Regulation & Performance during Spaceflight

PI: Charles Czeisler, Ph.D, M.D.

Brigham and Women's Hospital/Harvard Medical School

Research Focus: Synchronization to Mars' day with two brief light pulses

Specific aims:

Test the hypotheses that:

1. Synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~4% longer than its intrinsic period will be disturbed.
2. This disturbed circadian synchronization will disrupt sleep, endocrine function, and impair waking alertness and performance.
3. Two relatively brief (45 minute) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase in subjects on such a schedule, resulting in improved sleep consolidation, undiminished growth hormone and cortisol secretion and enhanced daytime alertness and performance.

Research Progress 11/1/2002-10/31/2003:

We have designed a 65-day long between subjects randomized clinical trial to test the three hypotheses. Twelve subjects were randomly assigned to either Cell A, or B, or C. Subjects assigned to Cell A and Cell B were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 and 100 lux in the angle of gaze during wakefulness, respectively. Subjects assigned to Cell C were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 to 100 lux in the angle of gaze during wakefulness, but in addition, they were exposed to two brief 45 minute pulses of bright light (approximately 10,000 lux) in the evening, separated by 1-h of approximately 100 lux, as a countermeasure to entrain their pacemaker to the longer-than-24-hour day.

During FY03 we completed the last two experiments needed to complete the study (12 subjects studied for 65 days each in total). Data collected include: Core body temperature, blood samples (melatonin), Urine samples, Sleep and waking EEG recordings, Subjective sleep quality, Actigraphy, Light intensity, neurobehavioral performance and mood. Data analyses are currently in progress. The successful collection of the data will allow us to test hypotheses 1, 2, and 3 of the project. Plasma samples have been analyzed for melatonin, and period, circadian phase and phase angle of entrainment have been determined for all subjects throughout their 65-day study. Temperature data have been edited and also analyzed for estimation of intrinsic circadian period. Data analyses are currently in progress for polysomnographic parameters (sleep and waking EEG), as well as performance tests and mood questionnaires. Preliminary data have been presented in two major meetings (Gordon Conference in Italy, and American Society for Photobiology in Baltimore, MD). A manuscript is currently in preparation; its submission for publication is anticipated this coming year.

We anticipated that subjects in cell A and B would fail to appropriately entrain their circadian pacemaker to the longer-than-24-hour day, resulting in sleep and endocrine disruption and impaired alertness and performance. We anticipated that subjects in Cell C, that is, scheduled to receive two relatively brief (45 minutes) daily exposures to evening bright light (approximately 10,000 lux) as a countermeasure to circadian misalignment, would establish a normal entrained circadian phase.

Our preliminary results indicate that subjects exposed to the two evening episodes of bright light demonstrate entrainment of their melatonin rhythms to the imposed sleep-wake cycle. They also reveal that lighting levels of approximately 25 lux (comparable to the intensities measured in the middeck for STS-95) were insufficient to maintain a normal phase angle of entrainment, and that approximately 100 lux (comparable to the intensities measured in the Spacehab for STS-95) was also insufficient to maintain a normal phase angle of entrainment in all subjects. Therefore, our preliminary results indicate that two relatively brief (45 minutes each) exposures to bright light in the evening are effective in maintaining entrainment of the circadian pacemaker to longer-than-24 h days, and would be effective as a countermeasure to circadian misalignment during space flight. Our data also suggest that ordinary room light (100-150 lux in the angle of gaze) might be sufficient to maintain entrainment to the 24.65-h solar day of Mars in a small number of astronauts, although at an abnormal phase angle that would be expected to induce sleep disturbance. Both the model and our preliminary data indicate that those astronauts who will have the greatest difficulty adapting to the 24.65 h Martian day will be those with an endogenous circadian pacemaker that has a period shorter than 24 h, which we estimate represents approximately 25 per cent of the population.

Optimal human performance during space flight requires astronauts to maintain synchrony between the circadian pacemaker which regulates the timing of sleep, endocrine function, alertness and performance and the timing of the imposed sleep-wake schedule. Operational demands of space flight necessitate that humans live on day lengths different than the 24-h solar day of Earth. Due to orbital mechanics, astronauts are commonly scheduled to the near equivalent of a shorter-than-24-hour day length in Earth orbit on space shuttle missions; moreover, they will be scheduled to the 24.65-h solar day of Mars on the planned exploration class mission to Mars. Through current support from the NSBRI and NASA, we have demonstrated that a scheduled dim light-dark rest-activity cycle, with a dim ambient light intensity similar to that used aboard the space shuttle middeck, is able to entrain most, but not all human subjects to a scheduled 24-hr day, whereas none of the human subjects scheduled to a 24.6-h day (the period of the axial rotation of Mars) were entrained to this weak synchronizer. Circadian phase misalignment to the 24.6-h day resulted in sleep disturbance (reduced sleep efficiency), endocrine disturbance (secretion of the sleep-promoting hormone melatonin during the waking day, reduced nocturnal growth hormone secretion and reduced cortisol levels), and impaired daytime alertness and neurobehavioral performance (reduced vigilance). The degree of circadian misalignment to the 24.6-h day was found to be strongly dependent upon the period of each subject's circadian pacemaker, such that subjects with periods shorter than 24.0 hr demonstrated the greatest degree of circadian misalignment to the 24.6-h day. Due to concerns over high radiation exposure during the voyage to Mars and while on the planet's surface, NASA engineers have indicated that neither the spacecraft nor the Martian habitat may have windows. Our data suggest that most if not all astronauts would exhibit circadian misalignment if the space flight lighting conditions of approximately 10 lux on the windowless middeck of the space

shuttle were present on the space craft while en route to Mars or on the Mars station during their approximately 540 day stay on Mars. The data collected during the past four years of support from the NSBRI demonstrate the need to develop effective and attainable countermeasures to prevent circadian misalignment during an exploration class mission to Mars. Our preliminary results suggest that exposures to bright light episodes of relatively short duration are effective in resetting the human circadian pacemaker and may provide an effective countermeasure to prevent circadian misalignment during exploration class space missions. This work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dyssomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions. Careful analysis of the efficacy of brief, intermittent bright light episodes in the treatment of these conditions has important ramifications for the practical application of bright light treatment during space flight, since repetitive uninterrupted exposure to bright light for many hours each day would not be feasible due to the cost of generating power and other operational demands of space missions. The results of our research could have a profound effect on the health, productivity and safety of astronauts during an exploration class mission to Mars. On earth, this work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dyssomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions.

Project 3: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

*PI: David F. Dinges, Ph.D.
University of Pennsylvania*

Research Focus: The use of naps to ameliorate the affects of chronic sleep restriction

Specific aims:

1. Establish Response Surface Map to determine how to best use anchor and nap sleep periods to promote neurobehavioral performance and alertness at an adverse circadian phase for waking;
2. Identify the optimal diurnal anchor sleep and nocturnal nap schedule to maintain neurobehavioral function when work is initiated with abrupt circadian displacement;
3. Determine how diurnal anchor sleep times and nocturnal nap sleep affect sleep physiology and circadian adjustment across a chronic schedule of simulated night operations.

Research Progress 11/1/2002-10/31/2003:

This project specifically addresses the following questions in the Human Performance Factors, Sleep and Chronobiology research area:

1. Which countermeasure or combination of behavioral and physiological countermeasures will optimally mitigate specific performance problems associated with sleep loss and circadian disturbances during a Mars mission? (Bioastronautics Critical Path Roadmap question 6.06)
2. What are the acute and long term effects of exposure to the space environment on biological rhythmicity on sleep architecture, quality and quantity, and their relationship to performance capability? (Bioastronautics Critical Path Roadmap question 6.05)
3. What workload schedule(s) per workday will best enhance crew performance and mitigate adverse effects of the space environment? (Bioastronautics Critical Path Roadmap question 6.10)
4. What are the long term effects of countermeasures employed to mitigate performance problems with sleep loss and circadian disturbances during a Mars mission? (Bioastronautics Critical Path Roadmap question 6.07)
4. What are the long-term consequences of the use of countermeasures designed to mitigate performance decrements associated with sleep loss and/or circadian disturbances? (NASA/NSBRI Critical Path question 6.07.)

To date we have completed the study of N=48 subjects in this protocol. Analysis of the polysomnographic, neurobehavioral and neuroendocrine data is underway on the data collected from the completed subjects, so that we may integrate this data into our existing response surface models. In experiment 1 we have been using a response surface experimental approach to systematically determine the chronic (10-day) effects of 18 sleep schedule conditions that involve restricted nocturnal anchor sleep alone and in combination with varying durations of restricted diurnal naps on performance, mood, sleep, circadian physiology and hormones. The resulting preliminary response surface maps (RSMs) derived from this dose-response experiment indicate that total sleep time per 24hr is a prime determinant of cumulative neurobehavioral deficits, and that combining a restricted nocturnal anchor sleep with a midday nap can attenuate cumulative deterioration in performance. In order to complete our understanding of how to

optimize performance in the face of restricted sleep in space flight, in the current experiment we have reversed the circadian placement of these 18 anchor sleep + nap sleep conditions (i.e., diurnal anchor sleep alone and in combination with varying durations of restricted nocturnal naps), in order to (1) establish the RSMs during simulated night operations; and (2) by comparison with the RSMs for the study currently being completed, to determine the role of initial circadian phase of sleep on the cumulative rate of impairment from chronic sleep restriction. Response surface maps will be generated when the sample size is 75% complete, but descriptive analyses of the data obtained thus far supports the findings from experiment 1. That is, achieving physiological sleep on split-sleep schedules was possible, and naps helped attenuate some cumulative cognitive impairments—especially in working memory—but total sleep time per 24h remained the prime determinate of vigilance and behavioral capability over time. Moreover, naps taken at the nocturnal circadian phase, resulted in severe sleep inertia upon awakening, which had to dissipate before nap benefits on performance were seen.

Project 4: Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate.

PI: Russell Foster, Ph.D.

Imperial College, London, UK

Research Focus: The impact of non-rod, non-cone ocular photoreception on physiology and behavior.

Specific aims:

1. To determine the role of novel photoreceptors in the regulation of general physiology and behavior. The experiments in this section will determine the extent to which body temperature, heart rate (ECG) and EEG are modulated by non-rod, non-cone ocular photoreceptors using a unique mouse model (*rd/rd cl*), which lacks all rod and cone photoreceptors.
2. To define the relationship between novel ocular photoreceptors, light, sleep state and levels of *c-fos* expression in the ventrolateral preoptic nucleus (VLPO) of the brain. The VLPO integrates light information to modulate sleep state. We will establish whether the light information that reaches this brain structure is primarily from the rods, cones, novel receptors, or a combination thereof.
- 3) To characterize the molecular mechanisms of non-rod, non-cone ocular photoreception. Three broad strategies will be undertaken to look for these molecules based upon bioinformatics, proteomics, and micro array technology. Our aim will be to identify a set of genes that: a) share sequence similarity with proteins known to be involved in photoreceptor/sensory cell function; b) are expressed in light sensitive cells of the inner retina; c) undergo post-translational modification and/or changes in expression following light exposure. We will subsequently confirm that these genes are expressed in candidate photoreceptive cells using *in situ* hybridization, single cell PCR from photosensitive retinal neurons, and the generation of suitable knockouts where appropriate.

Research Progress 11/1/2002-10/31/2003

The long-term aim of this project is to provide effective countermeasures for the detrimental effects of the irregular and altered patterns of light exposure in space and on the surface of other planets. These countermeasures will depend upon an understanding of the basic mechanisms of how the eye perceives light. The eye has been one of the best-studied parts of the central nervous system and its fundamental functions were considered well understood. All photoreception within the eye of mammals was considered the province of the rods and cones. However, studies over the past decade have shown that varied aspects of physiology and behavior are regulated by gross changes in environmental light, and that these irradiance changes are detected by novel ocular photoreceptors. The photoreceptive mechanisms underlying these, non-rod, non-cone photoreceptors remain uncharacterized. Yet their importance in our daily lives is profound. It is now clear that light exposure can influence alertness and sleep propensity. Furthermore, light regulates the phase of circadian clocks, and thus the timing of rhythmic functions such as digestion, sleep and performance. The central aims of the research to be undertaken at Imperial College are to characterize the molecular mechanism of this unexplored photoreceptor system of the eye, and determine the extent to which these photoreceptors contribute to varied aspects of

physiology and behavior. In this regard our work spans 1-4 of the Countermeasures Readiness Levels (CRL) of NASA's Biomedical Research and Countermeasures Program. By employing a unique rodless+coneless mouse model (rd/rd cl), and taking advantage of the new opportunities created by a range of post-genomic technologies, including the imminent completion of sequences for both mouse and human genomes, we will address our specific aims (above). The results from these integrative studies will provide the mechanistic substrate for both targeted drug development aimed at the manipulation of human circadian rhythms, sleep, mood and performance, and the design of new lighting sources that are either highly effective in regulating these novel photoreceptors or leave them largely unstimulated.

Although the project did not formally start until the 1st of October 2003, we have anticipated the commencement of funding and: (i) established the breeding lines of mice required to undertake the proposed work; (ii) put into place most of the telemetry equipment; (iii) have generated preliminary data relating to our third aim and. (iv) initiated the microarray experiments to determine patterns of light-induced gene expression in mice lacking rods and cones. We also have shown that the ventrolateral preoptic nucleus (VLPO) shows light-induced Fos expression in rodless+coneless mice which suggests that sleep state is indeed modulated by non-rod, non-cone photoreceptors.

Project 5: Primate Circadian Rhythms in the Martian Environment

PI: Charles A. Fuller, Ph.D.
University of California, Davis

Research Focus: Bright light pulses, Period vs. Gravity

Specific aims:

Test the hypotheses that:

1. Rhesus macaques will not entrain to the Martian solar day when exposed to ambient light available on Mars, resulting in performance decrements and sleep and circadian dysfunction;
2. Some, but not all rhesus macaques will be able to entrain to a Martian solar day under a lighting environment proposed for the Martian Habitat;
3. The rhesus circadian period will change as a direct function of G level in hypergravity;
4. Daily evening pulses of bright light will synchronize all rhesus monkeys to the proposed Habitat environment

Research Progress 11/1/2002-10/31/2003:

1. Effect of Gravity on the Circadian Period of Rhesus Monkeys

During this time the study of circadian period at 2G was completed using our forced desynchrony protocol. Determination of circadian period at 1G was repeated with a replacement 2G animal. We are reviewing the usability of the 1.5 G data, however comparison of periods in 1 and 2G showed a trend toward decreased circadian period with increased gravity, the opposite of the relationship hypothesized based on insect studies. At 1G circadian periods ranged from 24.0 to 25.33h in five animals (mean 24.62h, SE 0.31). Circadian periods in 2G ranged from 24.12 to 25.05h (mean 24.51h, SE 0.023). This trend was not statistically significant, and we will increase our sample size this Fall when female rhesus are studied using the same protocol. The female rhesus candidates have been selected and trained to the Psychomotor Test System (PTS) and we anticipate completion of the telemetry implant surgeries by 10/31/2003. Thus, we will be able to begin this study in November. Evaluation of sleep and performance data from the male centrifuge study is ongoing. The male centrifuge study yielded one novel finding for a primate. Several of the animals were unable to entrain to a 24-h light-dark cycle when in 1.5 and 2 G. This lighting condition effectively entrains the animals at 1G.

2. Acclimation to Martian Day Length and Altered Lighting Environments

Studies of Martian day length were completed for female rhesus using both blue-green deficient light (Mars simulation) and light with a solar spectrum. In both lighting conditions the rhesus were able to entrain to the Martian day (24.66 hours). We elected to determine the endogenous circadian period of the animals to determine how great a daily adjustment was required to align their circadian rhythms to the longer external day. Since successful entrainment eliminated the opportunity for countermeasure evaluation, we initiated a forced desynchrony study using the

Martian lighting simulation at the equivalent photon density of 100 lux daytime illumination. In these conditions circadian periods in the female rhesus ranged from 24.38 to 25.77 hours, the latter being atypical of the rhesus. The longest daily phase delay required for entrainment was thus 0.28 hours and the longest phase advance 1.11 hours. Evaluation of sleep and performance for the female rhesus is ongoing. The male rhesus have been selected, PTS-trained, and implanted with biotelemetry transmitters. We have initiated acclimation and baseline data collection, and anticipate beginning the first study in the Martian day on 11/21/2003.

Significance of Findings

The relationship of circadian period to G level seen in the male rhesus suggests that circadian period may on average shorten with increased G. We will need to repeat this study with the female rhesus, however, before this can be concluded. If this is the case, it would complement indications from space flight that circadian period is lengthened in mammals with decreased G. These studies also provided the first suggestion that light sensitivity of the circadian timing system may be reduced in altered G. Previously we have only demonstrated this in rodents. Reduced light sensitivity in space flight might also be hypothesized and would be consistent with impaired circadian entrainment.

Similarly, we will need to repeat the studies of entrainment to the Martian day and lighting simulation with the male rhesus before we can conclude that entrainment to these conditions is easily accomplished. The range of circadian periods seen in the particular female rhesus studied tended to be long compared to our previous findings in rhesus. We expect that at least some of the males will have shorter circadian periods and thus will be more hard pressed to entrain to the longer external day. Our findings are nevertheless encouraging and tend to suggest that with the proper lighting entrainment of human crews to the Martian day might be feasible.

Project 6: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

PI: Megan E. Jewett, Ph.D.

Brigham and Women's Hospital/Harvard Medical School

Research Focus: Use of light and performance mathematical models in schedule design

Specific aims:

1. Further develop and refine the dynamic stimulus processing model by using data from existing four studies;
2. Perform validation analyses on the revised models;
3. Incorporate these refinements into models of neurobehavioral performance;
4. Develop a user-friendly predictive performance software program that can be used in-flight as a self-directed countermeasure.

Research Progress 11/1/2002-10/31/2003:

Specific aims proposed in this project are : Aim 1: To further develop and refine our Light Model using data from four studies of the effects on the human circadian system of different stimulus cycles of brief or extended and bright- or moderate-intensity light pulses. Aim 2: To validate the Light Model refined above in Aim 1 using data from four different studies of the effects on the human circadian system of single-cycle patterns of brief or extended bright light pulses and of sleep-wake/light-dark schedules with a wide range of periods. Aim 3: To incorporate the Light Model into our Neurobehavioral Performance Model and validate this model using data from the above 8 studies. Aim 4: To develop a user-friendly Circadian Performance Simulation Software (CPSS) for field applications.

We have finished the analysis outlined in Specific Aim 1 that incorporates a dynamical and statistical method for extracting the amplitude recovery dynamics of the human circadian pacemaker. We have used this process to compare the two mathematical models that currently exist to explain the effect of light on the human circadian pacemaker. Our results demonstrate that the model with the higher order equation fits the amplitude recovery dynamics seen in core body temperature (CBT) data better than the model with the lower order equations. This higher-order model has been incorporated into our user friendly Circadian Performance Simulation Software. A manuscript of these results is in progress.

Members of our team, with collaborations from Dr. Laura Barger (Dr. Czeisler's group), have participated in a working group focused on setting space station schedule shifting guidelines. Our contribution to the working group was to apply our user-friendly Circadian Performance Simulation Software (CPSS), Version 1.2 to simulate current as well as proposed schedule shifting guidelines for crews on space missions. CPSS was used to evaluate the circadian performance and alertness of a particular schedule, where light exposure could be simulated as a countermeasure to the schedule design. The findings of the working group were incorporated in a working document that was used to discuss shifting guidelines with the Russian Space Agency. This work was requested on behalf of NASA via the Wyle Laboratory.

In year three of the program, we have been focusing on refinement of our mathematical model for the effect of light on the human circadian pacemaker. We have found limitations in our

current model such that the model fails to predict preliminary results obtained in Project 2 of the HPFSC team regarding entrainment to the 24.65-hr Mars day, as well as published results on entrainment to the 24-hr day by a weak light stimulus in Wright et. al. 2001 PNAS. To improve predictions, we have introduced a non-photic component that incorporates non-photic stimuli as an input parameter to our mathematical model. Studies performed at the BWH support evidence that there may be a weak entrainment effect of the sleep-wake cycle (one type of non-photic stimulus) on the human circadian rhythm. In particular, it has been shown that a blind subject without conscious light perception could be entrained to a non-24-hr day by a strict sleep-wake schedule only. This refinement of our model will allow us to make better predictions of human alertness and performance for sleep-wake schedules that may be encountered in space. Additionally, this refinement to the model allows us to make better predictions of the response of the human circadian pacemaker to brief or extended and bright, moderate or low-intensity light pulses. A manuscript of these results is in progress.

The distribution of CPSS 1.2 has increased from 31 to 134 sites. Divisions within NASA that have requested our software include NASA Ames-Fatigue Counter Measure Group, NASA-Johnson Space Center, and through a subcontract with Wyle Laboratories (sub-contractor of NASA). In addition, CPSS has been requested by agencies including the Office of Naval Research, the Naval Submarine Medical Research Laboratory, the Naval Health Research Center, the Walter Reed Army Institute of Research, AFOSR, DARPA, NIOSH, the US Department of Transportation, the FAA, the US Coast Guard, the Federal Railroad Administration, and commercial airlines. The overall distribution of our simulation software is approximately: 10% industry, 30 % governmental agencies, 30% University-related research institution, and 30% to individuals.

Project 7: A Model of Circadian Disruption in the Space Environment

PI: Michael Menaker, Ph.D.
University of Virginia

Research Focus: The effect of meals, melatonin, exercise, and dark pulses on circadian dysphasia

Specific aims:

1. Evaluate the effects of constant conditions and of shift work schedules on both the maintenance of circadian rhythmicity in central and peripheral structures, and on temporal synchrony among them in a transgenic rat model system;
2. Ameliorate or prevent circadian dysphasia by manipulating meal timing, melatonin administration, forced exercise and short pulses of darkness.

Research Progress 11/1/2002-10/31/2003

During the past year we have focused our major efforts on the question of what happens to circadian organization—in particular, the relationships between central and peripheral oscillators—when animals are rendered behaviorally arrhythmic by chronic exposure to bright constant light. The disruption produced by this treatment (described briefly below) provides a baseline against which we will evaluate the resynchronizing effects of timed meals, melatonin pulses, and periodic exercise in the next phase of the study.

We made Per1-luc rats arrhythmic by chronic, long-term exposure to bright constant light. These animals were sacrificed at two times of day 12 hours apart: 05:00 and 17:00 (we used clock times since the animals were arrhythmic). We cultured SCN and several peripheral tissues and asked: (1) were the tissues rhythmic in vitro, and (2) if so, was the phase of rhythmicity related to the time at which the animals were sacrificed? We reasoned that if a tissue were rhythmic and the phase of the rhythm in vitro was independent of the time of sacrifice, that would indicate that the tissue had been rhythmic in vivo. If the tissue were rhythmic in vitro, but its phase was dependent on the time of sacrifice, two explanations were tenable: (1) the tissue had been arrhythmic in vivo, and rhythmicity had been initiated by the culture procedure, or (2) the tissue had been rhythmic in vivo, but its phase had been reset by the culture procedure. [The two explanations are not that different, since for the second to occur the action of constant light would most likely have been to reduce the amplitude of the oscillations, making it more susceptible to resetting]. If tissues were arrhythmic in vitro, we assumed that they had been arrhythmic in vivo.

All tissues were rhythmic in vitro (except heart, in which 14 of 17 cultures were arrhythmic). The phases of rhythmicity in SCN, pineal and pituitary were directly dependent on time at which the animals were killed, peaking either about 1 hour after the cultures were prepared (SCN), or 30 hours after culture preparation (pineal and pituitary). On the other hand, the phases of cornea, ovary, and femoral artery were not affected by culture time. These results suggest that rhythmicity in “central” structures, either neural or endocrine, is abolished or repressed by conditions that produce behavioral arrhythmicity, but rhythmicity in at least some peripheral tissues is largely unaffected.

We next examined the effects of short exposures (1 week) to bright constant light which did not cause behavioral arrhythmicity. The phase of rhythmicity in cultured pineal and pituitary from these animals was not affected by the time of culture preparation, in contrast to their response to the time of culture preparation from arrhythmic animals. On the other hand, the phase of SCN rhythmicity from behaviorally rhythmic animals treated for short times with bright constant light was dependent on the time of culture to the same extent as in our behaviorally arrhythmic animals.

Taken together these results suggest that within the circadian system there is a hierarchy of sensitivity to the effects of constant light. The SCN is most sensitive; its rhythms of gene expression are abolished or at least severely damped by exposure to a constant light regimen that does not even cause behavioral arrhythmicity. Central endocrine tissues (at least pineal and pituitary) are less sensitive; their rhythms are abolished or damped by long term exposure to constant light, but not by short term exposure. Most peripheral oscillators are unaffected even by long term exposure to constant light, and are therefore least sensitive to its effects.

An alternative explanation is that tissues are inherently differentially sensitive to resetting by some aspect of the culture procedure. That does not account for the different effects of long term vs. short term exposure to bright constant light on pineal and pituitary, however it could explain, at least in part, the effects of culture time on SCN phase, which is not affected by the duration of exposure to constant light. In our next experiments, we will attempt to reverse the effects of long term exposure to constant light by periodic feeding, rhythmic application of exogenous melatonin, or forced exercise, alone or in combination. If successful, these treatments should eliminate the phasing effects of culture time on in vitro rhythmicity in SCN, pineal, and pituitary. That result would suggest that such treatments would be effective countermeasures against circadian disorganization caused by the abnormal schedules of space flight.

Project 8: Circadian and Vestibular System Relationships

PI: Lawrence P. Morin, Ph.D.
State University of New York, Stony Brook

Research Focus: Three dimensional motions to stimulate the vestibular and circadian system

Specific aims:

1. Identify efferent and afferent anatomical connections between the vestibular nuclei and the intergeniculate leaflet;
2. Test the hypothesis that patterned moving light (an optokinetic stimulus) will functionally activate the vestibular and circadian systems;
3. Test the hypothesis that a non-locomotor, non-photic three-dimensional motion stimulus will functionally activate the vestibular and circadian systems, as measured by FOS induction in the IGL and circadian phase shifts.

Research Progress 11/1/2002-10/31/2003:

Interactions between the circadian and vestibular systems: We have hypothesized that angular and linear acceleration data is transmitted from medial vestibular nuclei afferents to the IGL and the SCN, and are capable of acting as non-photic modulators for circadian behaviors. If true, this hypothesis would imply the existence of a vestibular-circadian psychophysical function which could help develop techniques for amelioration of chronobiological upsets such as jet lag or insomnia via specific vestibular stimulation. To examine these hypotheses, we are carrying out neural tract tracing studies looking for afferent and efferent interconnectivity between vestibular and circadian control nuclei, as well as examining FOS expression in these nuclei after different types and degrees of vestibular stimulation in a nocturnal rodent with a well-studied circadian system, the Syrian hamster (*Mesocricetus auratus*).

We have completed the following procedures: (a) Injection of the IGL with cholera toxin B subunit (CTb), a high quality retrograde tracer; (b) Injection of the MVe with Phaseolus vulgaris leucoagglutinin (PHAL), a high quality anterograde tract tracer; (c) Injection of the SCN with a transsynaptic tract tracer, PRV/gfp; and (d) Injection of individual animals with both CTb in the IGL and PRV/gfp in the SCN. The injections are complete and nearly all the data have been analyzed.

We are presently in the final stages of completion of a large manuscript describing the sources of ventral midbrain and hind brain innervation to the IGL. Briefly, CTb injected into the IGL has identified about 12 brain regions in the non-visual midbrain containing neurons that project to the IGL. These include the dorsal raphe and locus coeruleus regions that generally provide innervation to much of the brain. Others are peripenduncular, Barrington's, parabrachial, retrorubral, pedunculopontine tegmental, subpeduncular tegmental, lateral dorsal tegmental, lateral dorsal tegmental (ventral), cuneiform and the deep mesencephalic nuclei; nucleus of Darkschewitsch, supraoculomotor periaqueductal gray, and nuclei in the vestibular complex. Intraocular tracer injection has identified visual projections to the parabrachial nucleus. With the exception of the dorsal raphe and locus coeruleus, all the nuclei identified with CTb are involved in some fashion or other with eye movement regulation. Use of the PRV/gfp identifies neurons in the various pathways providing input to the SCN. This tracer identified numerous neurons in the

IGL, as expected, but not in all the other regions (e.g., nuclei of the tectum and pretectum) that project to the IGL. This indicates the strong likelihood that the IGL contributes to the regulation of two or more functions, one related to eye movements and the other related to circadian rhythm control. In most of the regions with neurons projecting to the IGL, notably in the MVe, there are cells labeled by retrograde PRV/gfp applied to the SCN and by CTb applied to the IGL. In some animals injected with both tracers, individual cells in the MVe contain each tracer. Cells so identified tend to be scarce in all nuclei with neurons projecting to the IGL, except in the IGL contralateral to the site of CTb injection.

The results support the following conclusions: (1) Cells of the MVe project to the IGL; (2) Cells of the IGL project to the SCN; (3) Some of the MVe cells make multisynaptic connections with the SCN; (4) Some of the MVe cells appear to make multisynaptic connections with the SCN via a synapse with cells in the IGL; (5) The ventral lateral geniculate nucleus (VLG; immediately ventral to the IGL) has most, but not all, of the connections exhibited by the IGL (no cells in the cuneiform nucleus project to the VLG); (6) The large majority of regions containing cells projecting to the IGL and VLG have known function related to eye movement regulation; (7) The IGL is likely concerned with both circadian rhythm and eye movement regulation; (8) The systems regulating circadian rhythms and eye movements appear to be largely independent of one another. (9) Contribution of the IGL to eye movement regulation has not previously been demonstrated in any species.

FOS expression following vestibular stimulation: The FOS protein is a well studied marker for neuronal activation and has been used in numerous circadian and vestibular studies. The number of animals stimulated with linear (anterior-posterior or lateral shaking) or angular (rotation through the inter-aural axis) vestibular stimulation under dark conditions has been expanded to 52 hamsters. Differential FOS expression was noted among the vestibular nuclei based on stimulus condition. Animals subjected to linear acceleration at rates >1.0 Hz (>1 G at endpoints of movement) showed FOS expression in the SCN and IGL bilaterally. Animals subjected to angular acceleration at rates $>60^\circ/\text{sec}$ showed lateralization of FOS expression in the MVe and IGL, and approximately symmetrical distribution of FOS label in the SCN. Number of cells in the target nuclei displaying FOS expression under both conditions was positively correlated with magnitude of angular acceleration, although we have not yet determined the saturation plateau. Control (restrained) animals and animals subjected to low levels of angular or linear stimulation showed little or no FOS expression. These findings raise the questions of whether there is a "threshold" function for modulating circadian behavior based on vestibular-GHT input to the SCN, and whether there are modality-specific processing regions within the IGL.

Project 9: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

PI: Gianluca Tosini, Ph.D.
Morehouse School of Medicine

Research Focus: Use of melatonin to synchronize rhythms

Specific aims:

1. Determine the effect of long-term exposure to constant conditions on circadian rhythms.

Research Progress 11/1/2002-10/31/2003:

The main aim of our research is to investigate how long term exposure to constant conditions (dim light at the intensity of 0.5-1 lux) affects the circadian organization of the rat.

We have held animals (36 Wistar rats for each of the three experimental groups) in a room with constant dim illumination (< 1 lux) and monitored the locomotor activity for each animal by a computerized system. Then after 0, 5 and 60 days, we have sacrificed the experimental subjects by decapitation at six different times of the day (CT 4, 8, 12, 16, 20 and 24). Tissues (SCN, pineal, retinas, lung, liver, and skeletal muscle) were collected from each animal, immediately frozen and then stored at - 80 °C. mRNA levels, in the SCN were determined by quantitative in situ hybridization, while mRNA level in the other tissues, were determined by real time quantitative PCR. The expression of the gene *Period1* was used as a marker of the phase of the circadian clock, while the expression of the arylalkylamine N-acetyltransferase (*Aa-nat*) gene was used as a marker of the phase of melatonin synthesis.

In LD conditions, *Aa-nat* mRNA levels in the retina showed marked day-night variations. *Aa-nat* mRNA rhythm persisted after 5 days of CDL conditions. After 60 days in CDL, in some animals, *Aa-nat* mRNA levels were found to be high during the subjective day or low during the subjective night suggesting that in these animals *Aa-nat* transcription was desynchronized with respect to the locomotor activity rhythms.

In LD conditions, *Aa-nat* mRNA levels in the pineal showed a marked day-night variation. *Aa-nat* mRNA levels were very low during the day and higher at night. *Aa-nat* mRNA rhythms persisted after 5 days of CDL conditions. *Aa-nat* signals were observed throughout the day with highest level at CT16-20 and lowest at CT12. After 60 days in CDL *Aa-nat* mRNA levels - in some animals - were high during the subjective day or lower during the subjective night.

Period1 expression in liver was rhythmic in LD and after 5 days in CDL. Long-term exposure to CDL did not significantly affect the patten of *Per1* expression in the liver. In the skeletal muscle *Period1* mRNA expression was rhythmic in LD and after 5 days in CDL. After 60 days in CDL - in few animals - *Period 1* mRNA levels were found to be desynchronized with respect to the locomotor activity rhythm. Analysis of *Period1* mRNA levels in the heart has not been completed yet.

Our work suggests that long-term exposure to constant condition has profound effects on the animal's temporal organization. In particular, our data demonstrated that in some animals (20-30 %) *Aa-nat* (and thus melatonin synthesis) rhythmicity in the pineal and in the retina is significantly affected by long-term exposure to CDL, similarly *Per 1* gene expression in the skeletal muscle was found to altered by long-term exposure to CDL in some animals.

Project 10: Animal Model for Sleep Loss and Circadian Disruption

PI: Fred W. Turek, Ph.D.
Northwestern University

Research Focus: The effects of chronic exposure to 12 hour periods of imposed wakefulness on sleep/wake, performance and circadian phase and the impact of countermeasures such as melatonin and exercise.

Specific Aims:

1. Determine the effects of 12 hours of imposed wakefulness during the normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measures in the mouse;
2. Treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle and/or neurobehavioral and motor performance measures;
3. Access to a wheel (exercise) when in the home cage, will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle and neurobehavioral and motor performance measurements.

Research Progress 11/1/2002-10/31/2003:

Our project addresses risk based goals one, two and three of the Human Performance Factors, Sleep and Chronobiology Team. Key findings during the past award year indicate that animals forced to be active either during the 12 hour light phase [normal rest period] or 12 hour dark phase [normal active phase] for 10 consecutive days are successfully chronically sleep deprived. Each day animals lost approximately 20-25% of their sleep. The degree of sleep loss seen in these studies is equivalent to a human obtaining 6 hours of sleep per night, which is commonly seen on shuttle missions. It also appears that there are differential effects on both the amount of sleep lost and recovered each day depending on the timing of the period of imposed wakefulness [light or dark phase]. These studies are the first to systematically partially sleep deprive mice for an extended period. An exciting aspect of the data collected during this unique study is that our results appear to be similar to those seen in data recently published on human subjects exposed to chronic partial sleep loss. This suggests that our unique animal model will be useful in developing effective new countermeasures that can be applied to humans. It also means that we will be able to successfully model various new or unusual schedules and conditions that humans may be exposed to during space flight or ground operations.

During the past year studies of chronic partial sleep loss during both the 12 hour light and dark phase, in two mice strains have been completed. Scoring and analysis of this huge data set is both a time and labor consuming process. Preliminary analysis of the data from C57BL6/J animals forced to be active during the light phase [normal rest phase] indicate a significant increase in wakefulness across the 24 hour day compared to baseline and recovery periods. During the periods of imposed wakefulness, mice never obtained REM sleep, but were able to get between 20-50% of baseline levels of NREM sleep. Upon release from imposed wakefulness each day [during the dark phase], mice responded with an approximately 4-5% increase in REM

sleep from baseline levels. In comparison, NREM sleep was only slightly higher in the dark phase, suggesting that NREM sleep was not recovered to the same degree as REM sleep. For each 24 hour period during the forced activity protocol there was less NREM and REM sleep than at baseline. Therefore, overall the animals were not able to recover all their sleep during the dark phase, resulting in approximately 26 hours of sleep loss across the 10 days of the protocol. During the recovery period REM and NREM sleep appeared to return to baseline levels on the first day of recovery. Analysis of the data from C57BL6/J animals forced to be active during the dark phase indicate a significant increase in wakefulness across the 24 hour day compared to baseline and recovery periods. Similar to animals in the wheel during the light, animals in the wheel during the dark, were not able to get any REM sleep but they were able to get some NREM sleep although less than at the baseline. During the light phase [normal rest period], wake, NREM and REM was similar to baseline levels. During the forced activity period during the dark phase [normal active phase] the amount of wake increased considerably and NREM and REM decreased. Overall the animals were not able to recover all their sleep during the light phase, resulting in 26.7 hours of sleep loss across the 10 days of the protocol. In addition, sleep returned to baseline levels on the first recovery day following the 10 days of imposed wakefulness protocol. Further analysis of this rich data set is required to elucidate the differences between groups. We will further analyze the architecture of the sleep periods to include variables that are indicative of sleep depth [delta power] and quality such as the number and duration of sleep bouts and sleep latencies to REM and NREM. Scoring and analysis of the sleep data from the B6C3F1/J (C3H) mice is ongoing.

In addition to examining sleep/wake, it was important to determine whether 12 hours of forced activity during either the light or dark phase altered circadian rhythms. Results indicate that the onset time of the core body temperature rhythm does not appear to be affected by the forced activity protocol under condition of high light levels. Analysis of this temperature data indicates that 12 hours of forced activity during either the light or dark phase did not shift the circadian rhythm of core body temperature compared to control animals for either strain of animal studied. However, there was an immediate effect of the forced activity on core body temperature. In addition, there did appear to be an increase in core body temperature at the end of the forced activity period when animals were placed back in their home cage. This is likely due to the animals participating in nesting behavior. Further analysis of the sleep data in comparison with the temperature data may allow us to address this question. In addition, relationship may not hold up at lower light levels.

Initial studies examining the impact of forced activity during the light phase on active shock avoidance performance indicate that there is no significant impact of sleep loss on this task when tested at the beginning of the light phase *after 12 hours of rest*. However, further experiments to examine performance following the forced activity protocol will be conducted to determine the impact of sleep loss on performance directly following the forced activity period when the animals will have had *no rest period*.

The key findings from the past ward year will allow us to further develop our animal model of sleep loss and circadian disruption. During the coming award year we will continue to analyze the data collected in the past ward year. In addition, with a clear baseline of sleep and circadian rhythms established we can determine further the impact of sleep loss on performance and the influence of the proposed countermeasures outlined in specific aim two and three. Initial studies involving a wheel and melatonin administration, will use the imposed period of wakefulness that

produces the most disruptive effects on the sleep-wake cycle and/or neurobehavioral and motor performance measurements.

OVERALL SUMMARY OF TEAM PROGRESS

The Human Performance Factors, Sleep and Chronobiology Team research progress continues to be driven by our three risk-based goals that are related to human performance failure because of sleep and circadian rhythm problems as identified in within Risk Areas 19 and 20 of the Critical Path Roadmap (2000). The team strategy is to work from a synergistic interactions between research projects at the molecular, cellular, organismic, and human clinical trial levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems. The team's research accomplished in the past year is intended to accelerate progress on countermeasures that reduce the risk of human neurobehavioral or physiological performance failure during space exploration. Specific countermeasures under study include lighting, napping, and non-photic interventions such as meal-timing, exercise, and melatonin administration. Aspects of habitat design (ambient lighting, space craft window specification, space suit visor design) and aspects of workload distribution and scheduling are focussed areas of significant progress towards effective countermeasures.

**NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
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I. ABSTRACT

During Fiscal Year 3 (2002-2003) of the present NSBRI grant, the Immunology, Infection, and Hematology (IIH) Team has developed a sharp focus on NASA Critical Path Roadmap Risk Areas and Critical Questions that deal with space radiation, microgravity, immunodeficiency, viral infection, and cancer. With the recommendations of the NSBRI External Advisory Council and NSBRI Board of Scientific Counselors in mind, the IIH Team has gathered research data, analyzed, and published its findings in over 30 reports in peer-reviewed literature. These publications describe gamma and proton irradiation of mice infected with a latent murine polyomavirus and the resultant combined detrimental effects upon the host ability to clear virus infection due to alterations in host immunity (reduced IFN- γ response to infection). Repetitive doses of radiation in this model resulted in the reactivation of polyomavirus infection. Human B cells irradiated in vitro also demonstrated harmful effects of gamma irradiation, with increases in the pro-apoptotic cell marker CD95 prior to cell death. Nutritional supplements reversed the lethal bacterial infectivity of mice in a microgravity model, and a green tea extract prevented attachment of the human retrovirus, HIV-1, to T lymphocytes. Human herpesviruses (EBV, CMV) and polyomaviruses (JCV, BKV) were documented in the blood and urine of control and immunosuppressed (HIV-1-infected) individuals by a sensitive PCR reaction as a prelude to studying astronauts. There was a reciprocal relationship documented, with decreased immunity leading to increased viral shedding. For the first time ever, SV40 (animal virus that contaminated early human measles vaccines) was detected in human lymphomas, an accomplishment recognized by the scientific world by a cover picture and key publication in *Lancet*. The study of gamma (^{137}Cs) and heavy metal ion (^{56}Fe) radiation on human hematopoietic stem cells and progenitor cells has been greatly facilitated by the adaptation of these cells' transplants into a mouse model that has severe depletion of B and T cells and NK cells (NOD/SCID/ β 2 mouse). Using this system, galactic cosmic rays (^{56}Fe) are significantly more inhibitory upon colony formation than gamma rays (^{137}Cs). In DNA damage and repair experiments, ^{56}Fe radiation again proved to be more harmful than other forms of radiation, and the Dicer gene that codes for the enzyme that converts double-stranded DNA into RNA is severely decreased. In novel experiments that combine the effects of space radiation (2 Gy gamma) and microgravity on a murine model, a synergistic effect of these two spaceflight conditions was observed in the number of splenocytes and thymocytes. In experiments designed to detect the effect of microgravity upon microbial genes, *E. coli* were observed to upregulate 18 genes including transcriptional gene regulators in the acid tolerance response (*yhiE*, *yhiF*), chaperone and associated gene (*hdeA*, *hdeB*, *hdeD*), cell motility genes (*flg*, *fli*), and chemotaxis regulating genes (*cheZ*, *tar*). Moreover, all of the 6 individual project leaders have submitted NSBRI competitive renewal applications in July of this year that propose to examine molecular mechanisms and cell lineage deviations in animals or isolated cells subjected to space radiation, microgravity, or the combination of radiation and microgravity. In addition, with the progress of the 6 currently funded projects and in the experiments proposed in the NSBRI competitive renewal grant applications, the Countermeasures Readiness Levels have risen from 2-3 (understanding scientific concept, proof of concept) to 5-7 (efficacy, clinical trials testing). The countermeasures being utilized or planned in future research include polypeptide inhibitors of caspase (enzyme mediating cell death), nutritional supplements, colony-stimulating factors for hematopoietic pluripotent stem cell lineages, opioid receptor blockers, and shielding for

radiation. The IHH Team Leadership is committed to promoting a strongly coordinated team effort, with collaboration of NASA scientists and external researchers that can extend the expertise and reach of the IHH Team.

II. INTRODUCTION

Background

Environmental factors that can influence the immune system include exposure to chemicals and elements, radiation, stress-inducing situations, and maintenance in conditions of isolation. All of these conditions can be found in spaceflight. In addition, spaceflight introduces the unique environmental conditions of exposure to altered gravitational fields and space radiation. In attempting to understand the harmful effects of conditions of spaceflight, it has become apparent that animals and humans become compromised in their ability to resist infections and the development of malignancy.

Rats flown in space were found to have hypoplasia of the thymus. Additionally, the proliferation of leukocytes was altered when tested after the rats returned from a US spaceflight. Interferon production by spleen cells taken from flight rats aboard the Space Shuttle for 7 days was reduced significantly, as compared with that by cells obtained from control rats. Alterations were observed in the following spleen cell populations from animals that had flown in space compared with those from ground-based controls: total T lymphocytes, CD8⁺ T lymphocytes, and interleukin-2 (IL-2) receptor-bearing T lymphocytes.

In cell populations obtained from crews immediately after return from space, the following results were observed: altered leukocyte subset distribution, altered production of IFN and other cytokines, altered natural killer (NK) cell activity, and diminished delayed hypersensitivity skin test responses to common recall antigens. Fifteen of twenty-nine Apollo crew members had difficulty with infections during and immediately after return from spaceflight. The infectious agents included influenza, *Pseudomonas aeruginosa*, and beta hemolytic streptococci. A more serious infectious disease incident occurred during the Apollo 13 mission. One crew member developed a urinary tract infection with *Pseudomonas aeruginosa* during the flight. Astronauts who have flown on the Space Shuttle have shown increased urinary catecholamine excretion and reactivation of Epstein-Barr virus (EBV). The observation that exposure to spaceflight could have led to alterations in immune responses that allowed the reactivation of latent viruses raises a concern with respect to susceptibility of spaceflight crew members to cancer.

In a series of experiments conducted at Loma Linda University (LLU), investigators carefully delineated the effects of proton radiation upon immune structure and function in murine models. Space or solar radiation is acknowledged to comprise photons (X-rays), electrons (gamma-rays), protons, neutrons, and heavy metal ions. C57BL/6 mice were given 3 Gray (Gy) protons in place of gamma-rays in one dose; some animals also received an immunization with sheep red blood cells (sRBC). By days 4 to 10 after irradiation, statistically significant decreases were found in CD19⁺ B cells, CD3⁺ T cells, CD4⁺ T cells, and CD8⁺ T cells. Irradiated animals showed a delayed and lowered anti-sRBC antibody response. Natural killer cells were relatively

radioresistant. B cells recovered by day 15 and CD3⁺ and CD4⁺ T cells by day 29, but CD8⁺ T cells remained impaired. Gamma radiation yielded similar data. The immunosuppressive cytokine, transforming growth factor- β 1 (TGF- β 1), was increased by day 7 in gamma-irradiated mice, but then fell in both proton- and gamma-ray treated animals by day 17. In a paired series of experiments in this murine model system, the LLU investigators found that gamma-ray treatment acutely (4 days) decreased spleen lymphocytes more than it did peripheral blood lymphocytes (PBL); higher doses were more toxic and acutely (4 days) decreased IL-2 secretion by activated splenocytes. These experiments provide ample evidence that space radiation is likely, at least acutely, to affect immune forces.

Space Health Concerns

In all human and animal subjects flown in space, evidence of immune compromise, reactivation of latent virus infection, and development of a pre-malignant or malignant condition exists. Moreover, in all ground-based space flight model investigations, evidence of immune compromise and reactivation of latent virus infection is also observed. All of these symptoms are similar to those found in a wealth of human pathological conditions where the human immune system is compromised, such as with stress, immunosuppressive drugs, infection, and radiation, and where reactivated, chronic virus infections and cancer appear as a natural consequence. Given these known risks to the immune system, it is highly appropriate, indeed imperative, that NSBRI researchers carefully investigate the effects of space flight conditions on human immunity, infection rate, and cancer rate.

Risks, Hazards, Major Critical Questions Identified in the Critical Path Roadmap

Risk #22: Immunodeficiency and Infections

- 7.03: *Do factors associated with flight (stress, environment, microgravity, nutritional status, radiation) affect humoral or cell-mediated immune function, non-specific immunity, mucosal immunity, or immune surveillance capabilities of crew members in a manner that exposes them to unacceptable medical risk (disease, allergy, delayed wound healing)?*
- 7.04: *Do factors associated with spaceflight increase activation of latent viruses?*
- 7.23: *Are there countermeasures for infections developed in space travel, especially latent virus infections?*

Risk #23 Carcinogenesis Caused by Immune System Changes

- 7.03: *Do factors associated with flight (stress, environment, microgravity, nutritional status, radiation) affect humoral or cell-mediated immune function, non-specific immunity, mucosal immunity, or immune surveillance capabilities of crew members in a manner that exposes them to unacceptable medical risk (disease, allergy, delayed wound healing)?*

7.04: *Do factors associated with spaceflight increase reactivation of latent tumor viruses?*

7.14: *What is the risk of cancer during or following long duration spaceflight?*

Risk #26 Altered Host-Microbial Interaction

7.06 *Does spaceflight alter microbial growth rates, mutation rates, or pathogenicity?*

7.09 *Does spaceflight alter the exchange of genetic material between microorganisms?*

7.11 *Does the spacecraft environment exert a selective pressure on environmental microorganisms which presents the crew with increased health risks (e.g., heliobacteria and ulcers)?*

7.13 *What diagnostic and environmental monitoring laboratory technologies need to be developed for the detection and diagnosis of infections in microgravity?*

7.23 *Are there countermeasures for infections developed in space travel, especially latent-virus infections?*

III. TEAM STRUCTURE AND DESIGN

Risks of spaceflight, (#22, 23, 26 listed in the Critical Path Roadmap, April 2003) have been reformatted into 5 risks for the team Strategic Plan:

1. Radiation Damage to Stem Cell and Immune System
2. Microgravity and Stress Effects on Immune System and Resistance to Infection
3. Reactivated Latent Infections
4. Malignancy
5. Altered Microbes

The fundamental mechanism that ties these risks together is the damage that produces the immunological deficits that create the observed risks (e.g., infections on space flights occur because of underlying immune damage).

The current projects of the Immunology, Infection, and Hematology (IIH) Team have come together in a synergistic manner (**Tables 1, 2**) to produce a unified program with significant discoveries and clear vision of where the team needs to go in the future. In essence, the team's goals are to: 1) reduce the risk of space flight conditions on bone marrow stem cells, and myeloid and lymphoid differentiated cells, 2) reduce the risk of astronauts developing new and reactivated viral infections, early cell death, and malignancy, and 3) reduce the risk of altered microorganisms and host-microbe interactions in space.

Table 1. Project Research Activities

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
BUTEL/Viral Infections and Mucosal Immunity	1-5	Pharmacological Agents	AOS; IR; Humans	Detect immune damage; Measure infection	Formulate antiviral reagents	
FOX/Microorganisms in the Spacecraft Environment	3-5	Pharmacological Therapy	Microbes	Develop microbe detection system	Perfect microbe detection system	Flight test detection system
GEWIRTZ/Effect of Deep Space Radiation on Human Hematopoietic Stem Cells	1,3,4	Stem Cell Therapy, Cancer Chemotherapy	In Vitro Stem Cells	Detect damage to stem cells	Formulate autologous stem cell transplant	Test stem cell Transplantation in space
SHEARER/Space Flight Immunodeficiency	1,3,4	Antibody Therapy, Stem Cell Therapy	IR; Humans	Measure apoptosis in thymocytes	Adapt Earth Rx strategies	Perform Rx in space
SHI/Endogenous Opioid-Mediated Fas Expression in Stress-Induced Lymphocyte Apoptosis	1,2,4	Cytokine Therapy	AOS, IR	Measure HPA in AOS, IR	Formulate drug treatment program	
SONNENFELD/Suspension, the HPA Axis and Resistance to Infection	2-5	Pharmacological Therapy	AOS, IR	Determine role of different stressors on immune responses	Formulate anti-stress program	Test program in space

Risks Key: 1) Radiation damage to stem cell and immune system; 2) Microgravity damage to immune system; 3) Reactivation of latent viral infections; 4) Malignancy; 5) Altered microbes

Definitions: AOS, anti-orthostatically suspended murine model; IR, irradiated mice; Humans, humans exposed to Antarctic winter or isolation in capsules; Microbes, microbial detection systems; HPA, hypothalamic pituitary axis; Rx, treatment

Table 2. Integration Activities

	BUTEL		FOX	GEWIRTZ	SHEARER	SHI	SONNENFELD
Internal Communication (E-mail, telecons, retreats, scientific meetings for all projects)	<ul style="list-style-type: none"> • Shearer • Sonnenfeld • Fox • Gridley (LLU) 	<ul style="list-style-type: none"> • Lugg (ANARE) • Pierson (NASA) • Larina (IBMP) 	<ul style="list-style-type: none"> • Butel • Sonnenfeld 	<ul style="list-style-type: none"> • Shearer • Shi • Sutherland (BNL) 	<ul style="list-style-type: none"> • Butel • Gewirtz • Fox • Shi • Sonnenfeld 	<ul style="list-style-type: none"> • Sonnenfeld • Butel 	<ul style="list-style-type: none"> • Shearer • Butel • Vazquez (Rad)
Integrated Experiment Development	Model Radiation and AOS Studies <ul style="list-style-type: none"> • Shearer • Gridley • Reuben • Sonnenfeld 	Model Radiation and AOS <ul style="list-style-type: none"> • Pierson • Larina • Lugg • Fox 	Collaborative Gene Probe Studies <ul style="list-style-type: none"> • Butel • Sonnenfeld 	Model Radiation Studies <ul style="list-style-type: none"> • Reuben • Shearer 	Model Radiation and Human Exposure Studies <ul style="list-style-type: none"> • Butel • Reuben • Lugg • Gridley 	Model AOS Studies <ul style="list-style-type: none"> • Gewirtz 	Collaborative Gene Probe Studies <ul style="list-style-type: none"> • Butel • Fox
Sample Sharing	Blood, Urine <ul style="list-style-type: none"> • Shearer • Reuben • Larina 	Microbes <ul style="list-style-type: none"> • Butel • Sonnenfeld 	Stem Cells <ul style="list-style-type: none"> • Reuben • Shearer 	Blood <ul style="list-style-type: none"> • Butel • Reuben • Dinges 	Blood <ul style="list-style-type: none"> • Butel • Reuben • Dinges 	Blood <ul style="list-style-type: none"> • Sonnenfeld • Butel 	Blood <ul style="list-style-type: none"> • Vazquez
Synergistic Studies of Opportunity	Antarctic Winter <ul style="list-style-type: none"> • Shearer • Lugg • Pierson 	Radiation, AOS <ul style="list-style-type: none"> • Butel • Sonnenfeld 	Radiation <ul style="list-style-type: none"> • Reuben • Shearer • Kennedy (Rad. Team) 	Antarctic Winter, Sleep Deprivation <ul style="list-style-type: none"> • Butel • Dinges • Lugg 	Radiation <ul style="list-style-type: none"> • Sutherland • Kennedy 	Radiation, AOS <ul style="list-style-type: none"> • Butel 	
Development of Computer Model of Integrated Human Function							

Definitions: ANARE, Australian National Antarctic Research Expedition; NASA, National Aeronautics and Space Administration; IBMP, Institute for Biomedical Problems, Moscow; LLU, Loma Linda University; Psych, Psychosocial Team; Rad, Radiation Effects Team; BNL, Brookhaven National Laboratory; AOS, Antithrostatic Suspension

Recently, the team determined that the major gap in our team research program was the absence of experiments with the space flight conditions of radiation and microgravity. The team has moved aggressively to fill that gap with experimental data published in the peer-reviewed literature. For example, the team published information describing enhanced morbidity and mortality of bacteria in small animals subjected to hindlimb-unloading model of microgravity.

In radiation-exposed animals, the amount of radiation (protons or γ - rays) absorbed by an astronaut going to Mars (3 Gy) acutely depresses both immune cells (myeloid and lymphoid cell types) and immune responses and alters the cytotoxic cytokine profile to render animals at risk of opportunistic infection. The mechanism for this loss of infection-fighting cells is likely due to apoptosis (early cell death). The team has begun to examine the DNA and gene microorganism patterns taken from animals subjected to these conditions of microgravity and radiation to determine if their resistance has been enhanced—creation of super bugs.

IV. TEAM RESEARCH ACCOMPLISHMENTS

Project 1: William T. Shearer, M.D., Ph.D., Principal Investigator; “Space Flight Immunodeficiency” (addresses Strategic Plan Risks 1, 3, 4 and Critical Questions 7.03, 7.04, 7.23, 7.24)

1. The Effect of Gamma Radiation on the Immune Responses of Mice with Polyomaviruses (Reuben, Butel, Shearer). Polyomavirus is a DNA virus that infects H-2(K) haplotype mice. Infection by this virus is usually cleared in 12 days, but it can lead to the development of tumors. PyV infection can be controlled with neutralizing antibodies, IFN- γ production, and cytotoxic T lymphocytes (CTL), with the latter two factors controlling tumor formation, as well. Four groups of 6 mice each were exposed to 3 Gy gamma radiation and/or 75 hemagglutinating units (HAU) of PyV in the following format: **Group A**, 3 Gy + PyV; **Group B**, no Gy + PyV; **Group C**, 3 Gy + no PyV; and **Group D**, no Gy + no PyV (total 24 mice). Replicate sets of mice were analyzed at 3, 10, 20, 49, and 200 days (total 120 mice). The salivary glands, kidneys, and spleen cells were examined for PyV DNA by PCR, and spleen cell supernates (Concanavalin-A [Con-A] stimulated) were evaluated for the presence of IFN- γ . **Figure 1** demonstrates the number of mice infected at each time point. Irradiation delayed the clearance of PyV. **Figure 2** demonstrates the level of IFN- γ production by splenic T cells.

Gamma-Irradiation Delayed Clearance of PyV Infection

Group	No. of mice determined by DNA PCR to be infected with PyV on days (d) post inoculation			
	d3	d10	d20	d49
A	6/6*	6/6	3/6	0/6
B	6/6	3/6	0/6	0/6

Groups: A= 3 Gy + PyV; B = PyV;
 *, PyV detected by PCR in at least 1 tissue

FIGURE 1

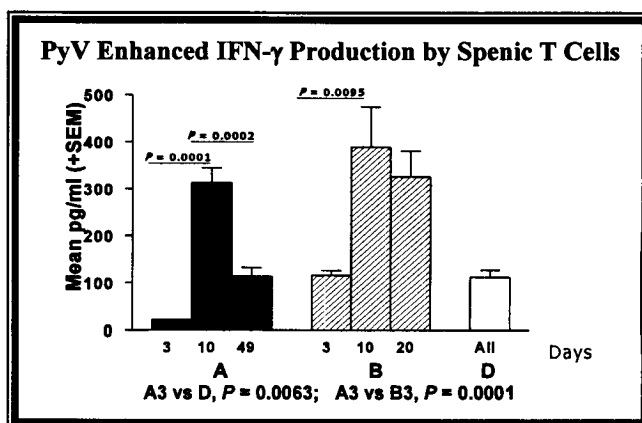


FIGURE 2

2. Reactivation of PyV with Repetitive Doses of Radiation: Effect of Complex Enhancers in Regulatory Region of PyV (Butel, Zhang). Since humans carry latent viruses from an early age and prior to exposure to space radiation, it is necessary to produce a murine model similar to the human experience. Short of infecting neonatal mice with PyV at birth, an alternate approach is to use repetitive doses of radiation after PyV infection. The regulatory region of PyV can be modified to contain simple (original nucleotide sequence) or complex (rearrangements of nucleotides) enhancers (**Figs. 3, 4**).

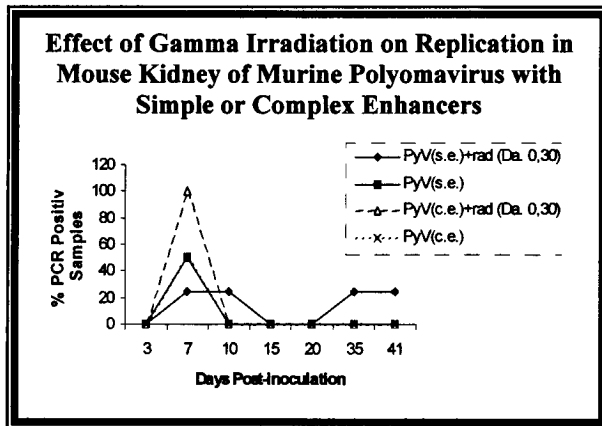


FIGURE 3

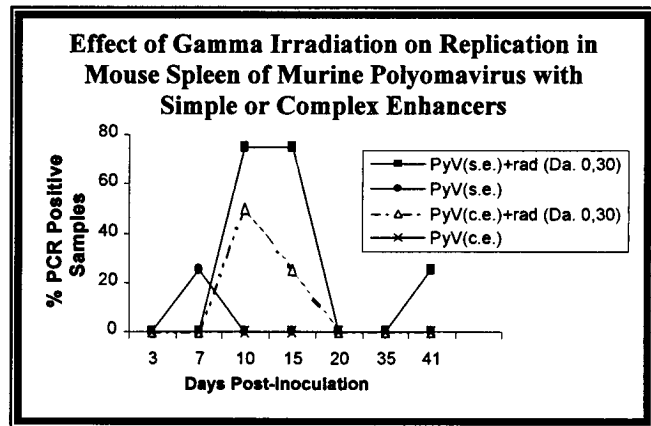


FIGURE 4

The data from this experiment fit the hypothesis that a better replicating virus is cleared more effectively by the host; that the more poorly replicating virus can escape immune elimination and establish persistent infections. In this experiment, the irradiated groups were both irradiated on day 0, virus inoculated on day 1, and irradiated a second time on day 30. There were 4 mice/time points; tissues tested were kidney and spleen.

In general, virus replication is elevated in samples from the acute phase of infection (first 2 weeks) from animals irradiated on day 0, compared to non-irradiated animals. Again, we detect some reactivation following a second irradiation (on day 30). **Figure 3** shows the results from the kidney tissues and **Figure 4** from the spleen. In both of those tissues, both simple and complex enhancer viruses disappear after about 15-20 days; following irradiation on day 30, only simple enhancer virus is reactivated. (Reuben, et al. 14th Space Radiation Health Investigators' Workshop, League City, TX, 2003; see **p. 15**).

3. Dendritic Cells and PyV Infection (Reuben, Lee). Dendritic cells (DC) are increasingly becoming recognized as perhaps the most important antigen-presenting cells (APC) in the generation of virus-specific CTL. There are two types of human precursor DC in the blood, and radiation has a negative effect on their maturation. Two subsets of murine precursor DC (pDC) that are homologues of the human blood plasmacytoid DC2 and myeloid CD11c⁺ DC1 precursors have been identified in the peripheral blood of mice. It is known that PyV infects DC, and exploration of this infection in our irradiated mouse model may be quite important for the development of a countermeasure (e.g., cytokine, Flt-3L, expansion of DC *in vivo*). Considerable investigation of human DC by one of the co-investigators (Dr. Reuben) will greatly facilitate the manipulation of DC in the mouse-radiation-PyV model. For example,

mobilization of human DC by granulocyte-macrophage colony-stimulating factor (GM-CSF) has led to an enlarged recovery of DC1 and DC2 from the peripheral blood (**Fig. 5**). Use of these DC-isolated populations has enabled the exploration of their utility as APC in presenting recall antigens [Candida (Can), StreptoKinase/Streptodornase (SK), tetanus toxoid (TT)] to autologous T cells (**Fig. 6**). We will be utilizing these techniques to assess the role of DC in mice infected by PyV and exposed to radiation in an attempt to discern the critical radiation- and virus-sensitive components of the immune response that clears virus infection. It may be possible to use Flt-3L (cytokine that expands DC populations) as a countermeasure prior to radiation to increase the number of DC.

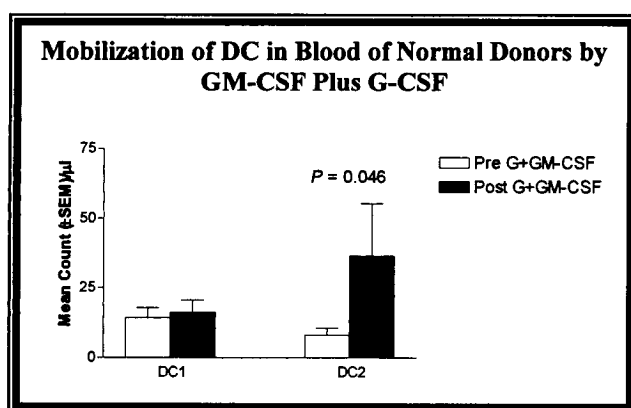


FIGURE 5

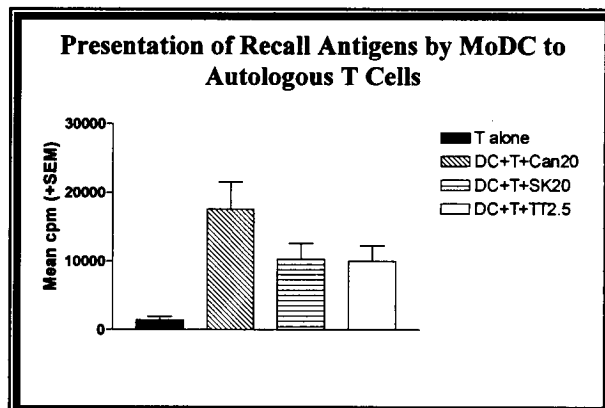


FIGURE 6

4. Human B-Cell Apoptosis in an Immunosuppressed (HIV-1) Model (Shearer, Nance). The HIV-1 envelope glycoprotein complex (gp120/gp41) is involved in both HIV-1-infected and HIV-1-uninfected cell death via its binding to cellular receptors CD4 and CXCR4. We studied the effect of HIV-1-envelope glycoproteins on apoptosis and the potential inhibitory mechanism of this process by stromal cell-derived factor-1 α (SDF-1 α), the natural ligand of CXCR4, in highly purified HIV-1 seronegative CD40-activated human B cells. Annexin V/propidium iodide (PI) (pro-apoptosis) staining revealed a significant (i.e., $p < 0.05$) dose-dependent increase in the percentage of apoptotic cells (annexin V-FITC⁺/PI) in the presence of HIV-1-gp120 (1-100 ng/ml). All HIV-1 envelope glycoproteins (100 ng/ml) tested, gp160, gp41, gp120, and gp120₄₂₅₋₄₃₆ (C2 binding region) induced significant increases of 93%, 114%, 172%, and 200%, respectively, on B-cell apoptosis. The presence of cleaved chromosomal DNA, as assayed by TUNEL, increased over ten-fold in the presence of HIV-1-gp120 (100 ng/ml). All other HIV-1 envelope glycoproteins tested produced similar results. Specificity of the HIV-1-gp120-induced B-cell apoptosis was demonstrated by the abrogation of effects by neutralizing anti-gp120 antibody but not by anti-CD4 antibody.

HIV-1-gp120 (1-1,000 ng/ml) increased Fas/APO-1 protein levels in highly purified HIV-1 seronegative donor CD40-activated human B cells in a dose-dependent manner ($p < 0.01$), and SDF-1 α reversed these increases ($p < 0.01$) (**Fig. 7**). HIV-1-gp120 also upregulated the pro-apoptotic Fas (CD95) receptor expression in human B cells that was reversed by SDF-1 α , again indicating activation of the Fas-mediated pathway. SDF-1 α -induced inhibition was blocked by either neutralizing anti-CXCR4 antibody or anti-SDF-1 α antibody. HIV-1-gp120 (100 ng/ml) significantly downregulated Bcl-2 (anti-apoptosis) expression and decreased Bcl-2 levels in CD40-activated human B cells. SDF-1 α (10-1,000 ng/ml) significantly augmented Bcl-2

expression and Bcl-2 levels (**Fig. 8**). Presence of either an anti-CXCR4 or anti-SDF-1 α antibody resulted in the abrogation of the SDF-1 α -induced apoptosis rescue.

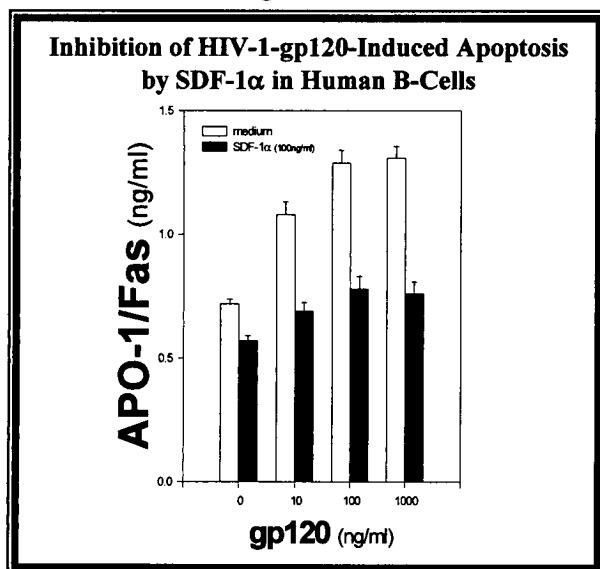


FIGURE 7

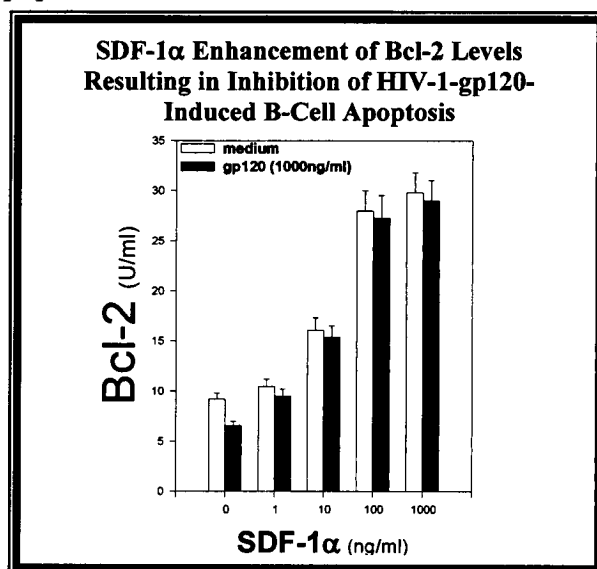


FIGURE 8

Studies measuring disruption of the mitochondrial transmembrane potential, an early event of apoptosis, revealed that CD40-activated B cells in the presence of HIV-1-gp120 (100 ng/ml) had significantly altered mitochondrial membrane potential, as determined by flow cytometric analysis utilizing a lipophilic cation as a mitochondrial activity marker. The addition of SDF-1 α (100 ng/ml) decreased this HIV-1-gp120-induced apoptosis. As activation of caspases is a hallmark of apoptosis, evidence of caspase activation as measured by D₂R-conjugated substrate cleavage showed increased caspase activation in the presence of HIV-1-gp120. D₂R substrate cleavage was not significantly altered in the presence of SDF-1 α .

The data suggest that the mechanism of HIV-1-induced B-cell apoptosis is mediated through the envelope glycoprotein, gp120, with the potential for SDF-1 α to reduce HIV-1-induced B-cell apoptosis through CXCR4-caspase-dependent signaling pathways and restoration of Bcl-2 function. It is possible that SDF-1 α may have therapeutic potential in the reduction of HIV-1-induced B-cell apoptosis. In the context of this research proposal, this carefully defined pathway of B-cell apoptosis serves as a platform upon which it will be possible to examine the effects of space radiation. Moreover, these findings will be used to investigate the effects of radiation on PyV-specific antibodies in the mouse model. (Nance, Shearer. Clin Immunol Suppl1:S111, 2003; see p. 14.)

5. Radiation Effects Upon Human B-Cell Proliferation, B-Cell Receptor Expression, and B-Cell Apoptosis (Shearer, Nance). As a proof of concept, we have begun a series of pilot studies with the human B-cell model described above to begin a systematic study of the effects of gamma radiation on receptor binding, signal transduction, and apoptosis. Isolated human B cells, activated by anti-CD40 monoclonal antibody and IL-4, were significantly altered by 3 Gy gamma radiation: proliferation was reduced by 70%, cell viability was reduced by 40%, CD79b (invariant component of the antigen receptor) surface marker expression was reduced by 50%, and the pro-apoptosis surface marker (CD95) was increased by 70% ($p < 0.01$) (**Fig. 9**), and the

anti-apoptosis surface marker (Bcl-2) was increased by 120% ($p < 0.01$) (**Fig. 10**). In these experiments, we exceeded the customary top dose of radiation (3 Gy) to determine if the trends seen at lower doses would be extended. At 6 Gy radiation, 50% viable cells remained. Results

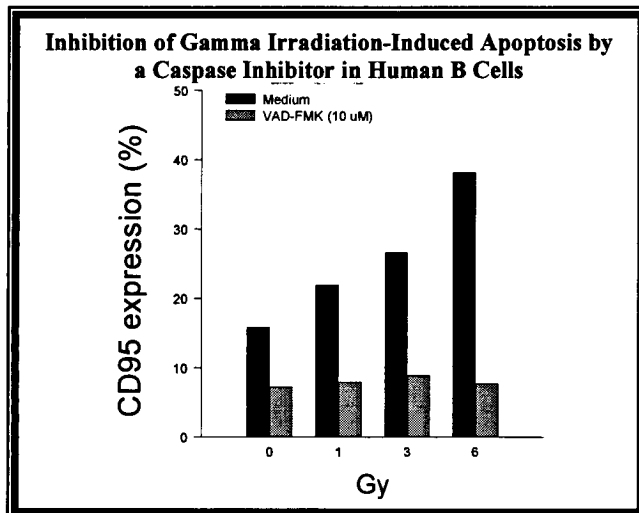


FIGURE 9

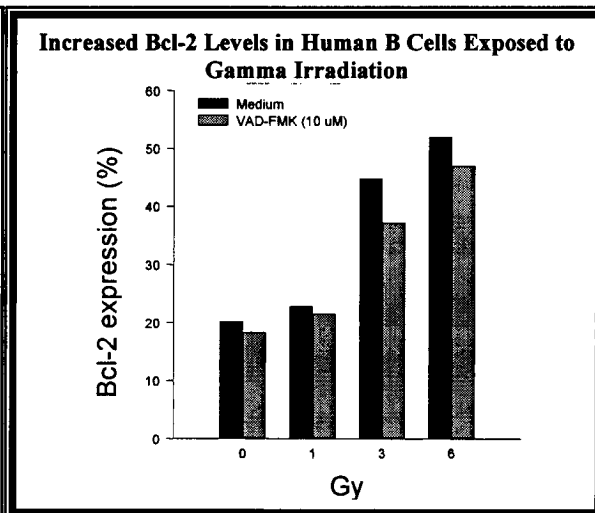


FIGURE 10

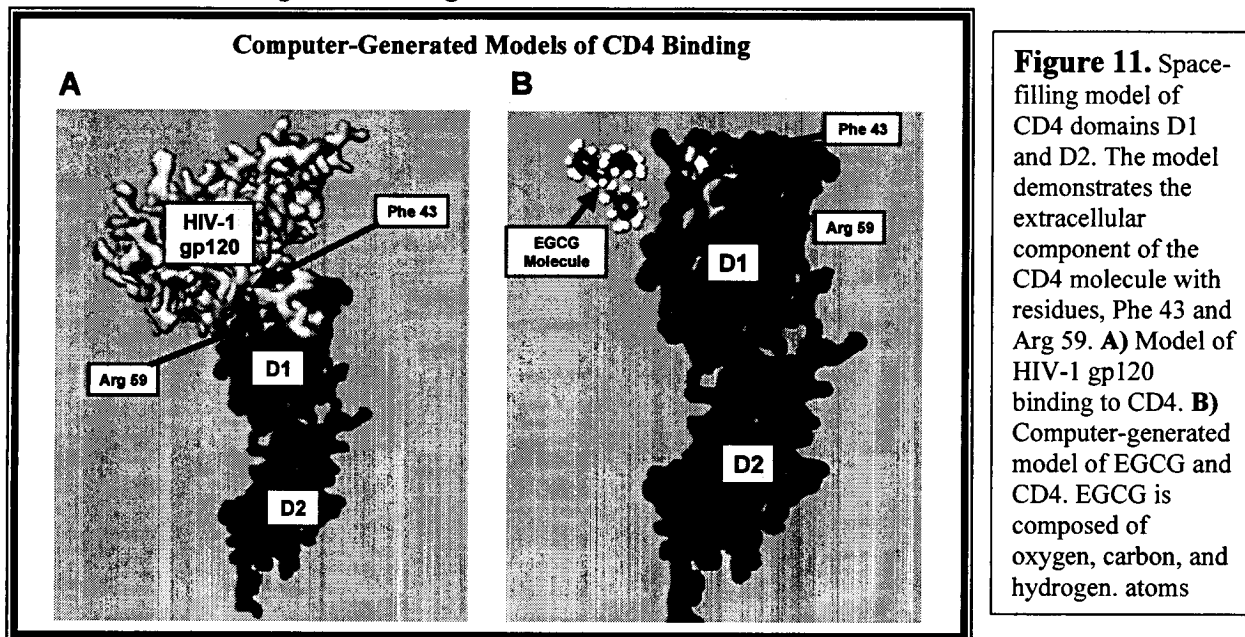
obtained in **Figures 9 and 10** are from viable cells.

As a first attempt as a countermeasures proposal, the general caspase inhibitor, benzyloxycarbonyl-valine-alanine-asparagine-fluoromethylketone (z-VAD-FMK), reversed the pro-apoptosis (CD95) marker while having little effect upon the expression of the anti-apoptosis (Bcl-2) marker expression (**Figs. 9, 10**).

Although extremely preliminary in nature, these pilot studies of irradiating isolated human B cells and then observing the differences induced in their behavior, under the previously defined receptor occupancy by ligands, metabolic changes, secretion of immunoglobulins, and pathways of apoptosis, promise to be a fruitful area of investigation. This model has the advantages of being human in origin, freshly harvested from donors (blood bank), and susceptible to space (gamma) radiation. (Nance, Shearer. 60th AAAAI Annual Meeting, San Francisco, CA, 2004 – J Allergy Clin Immunol, in press, 2004; see **p. 15**.)

6. Green Tea Polyphenol Epigallocatechin Gallate Binding to CD4 as a Model for the Inhibition of HIV-1-gp120 Binding to CD4+ T Cells (McCormick, Nance, Shearer). The catechin, epigallocatechin gallate (EGCG), the active component of green tea, has anti-inflammatory, anti-tumorigenic, and anti-oxidative as well as anti-viral properties. In HIV-1 infection, EGCG inhibits HIV-1 replication by the inhibition of HIV-1 reverse transcriptase with a resultant lowered p24 antigenemia. Recently, EGCG has been linked with interference of gp120 binding to CD4. We propose a model of EGCG binding to the CD4 molecule with competitive inhibition of HIV-1-gp120 binding (**Fig. 11**). Previously generated NMR spectroscopy and time-averaged nuclear Overhauser effects of EGCG protein chemistry were utilized in the development of multiple EGCG molecular conformations. Using the protein database file 1CDJ, the molecular structure of CD4 was illustrated via electron density mapping at 3Å. Modeling of EGCG binding to CD4 was achieved by computer-generated docking programs. Specific residues of the D1 domain of the CD4 molecule involved in HIV-1-gp120

binding were demonstrated to be potential sites of EGCG binding. Analysis of the residues involved in the binding of HIV-1-gp120 to the CD4 molecule revealed a binding pocket of amino acids, flanked by Phe43 and Arg59. The inherent conformational flexibility of EGCG lends itself to binding with this region of critical residues.



Mechanisms that interfere with the interaction of HIV-1-gp120 and CD4+ T cells have been purported as potential targets in HIV-1 treatment. We present a model of EGCG binding to a pocket of the CD4 molecule that might result in the interference of HIV-1-gp120 binding and subsequent inhibition of HIV-1 infection of CD4+ T cells. (Nance, Shearer. *J Allergy Clin Immunol*, in press, November 2003; McCormick, et al. 60th AAAAI Annual Meeting, San Francisco, CA, 2004 – *J Allergy Clin Immunol*, in press, 2004; see **p. 15**.)

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- Nance CL, **Shearer WT**. SDF-1 α regulates HIV-1-gp120-induced changes in CD79b surface expression and Ig production in activated human B-cells. *Clin Immunol* 105:208-214, 2002.
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- Sonnenfeld G, Butel JS, **Shearer WT**. Effects of the space flight environment of the immune system. Rev Environ Health 18:1-18, 2003.
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- Nance CL, **Shearer WT**. Is green tea good for HIV-1 infection? J Allergy Clin Immunol, in press (Nov, 2003).

Presentations: Abstracts/Lectures

- **Shearer WT**. Antarctica winter isolation alters the pro-inflammatory/anti-inflammatory cytokine balance in humans experiencing reactivation of latent viruses: implications for chronic viral infection and development of malignancy. NASA Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.
- **Reuben JM**, Butel JS, Lee BN, Zhang S, Shen DY, **Shearer WT**. The effect of gamma irradiation on the immune responses of mice with polyoma virus infection. 14th Space Radiation Health Investigators' Workshop, League City, TX, April 27-30, 2003.
- Nance CL, **Shearer WT**. HIV-1 envelope glycoprotein-induced B-cell apoptosis. Federation of Clinical Immunology Societies Meeting, Paris, France, May 16-19, 2003.
- **Shearer WT**. Antarctica winter isolation alters the pro-inflammatory: anti-inflammatory cytokine balance in humans experiencing reactivation of latent viruses: implications for chronic viral infection and development of malignancy. National Space Biomedical Research Institute Summer Teachers Program, Houston, TX, June 4, 2003.
- Nance CL, **Shearer WT**. Gamma radiation-induced human B cell defects: model for space flight. 60th Annual Meeting of the American Academy of Allergy, Asthma, and Immunology, San Francisco, CA, March 19-23, 2004, accepted.
- McCormick TG, Nance CL, Williamson MP, **Shearer WT**. Green tea polyphenol epigallocatechin gallate binding to CD4 as a model for the inhibition of HIV-1-gp120 binding to CD4+ T cells. 60th Annual Meeting of the American Academy of Allergy, Asthma, and Immunology, San Francisco, CA, March 19-23, 2004, accepted.

Project 2: Janet S. Butel, Ph.D., Principal Investigator; "Viral Infections and Mucosal Immunity" (addresses Strategic Plan Risks 1-5 and Critical Questions 7.03, 7.04, 7.14, 7.24)

All humans are infected for life with multiple latent and persistent viruses. In healthy individuals, pathogenic consequences are minimized due to control of virus replication or virus-

induced cellular proliferation by the host immune response, and it is well-known that suppression of the immune system allows latent viruses to escape control. The central hypothesis addressed by our project is that long-term space flight will damage the immune system, disrupting its ability to control viral infections and reactivations, resulting in virus-related diseases, including malignancy. We have focused our virus studies on human herpesviruses and polyomaviruses as these ubiquitous pathogens are known to establish latent infections in the human population and to undergo reactivation and cause disease when the host immune system is compromised.

Weather Report: Houston, the Texas Medical Center, and Baylor College of Medicine experienced extensive damage as a result of unprecedented rains and subsequent flooding associated with Tropical Storm Allison in June 2001. The storm represented one of the most intense rainfalls ever experienced in the Houston metropolitan area. Baylor and other institutions in the Medical Center suffered total power failures, flooding, and major damage to building infrastructures. Laboratories in the buildings which house this project were especially affected. Ongoing research encountered material losses and equipment failures; experiments in progress were lost; the vivarium biohazard space for infected animals was flooded and experimental animals lost. This disaster slowed research progress and data analysis on this project and delayed manuscript preparations. Nevertheless, we have succeeded in accomplishing the majority of what was proposed in the previous application and have used those findings to formulate the critical questions that need to be addressed in the next phase of this program.

1. Determine the Effect of Space Flight Conditions on Virus Reactivation, Infection, and Replication Using Ground-Based Models that Simulate Aspects of Space Flight.

a. Approach: There are several ground-based human models that mimic aspects of space flight (but not microgravity). One is wintering-over for 8-12 months in Antarctica; another is a closed chamber study in which individuals are confined within space-craft-like chambers on the ground. Both these conditions mimic, but do not precisely duplicate the stress, confinement, isolation, and microbial contamination expected to be encountered during actual space flight. To these we have added HIV-infected individuals, a medical condition in which patients suffer immunosuppression due to infection with HIV, the AIDS virus. HIV-infected individuals are the most extensively studied and best understood immunocompromised population. They are an effective model of medical problems that arise due to immune system damage and, by studying patients in various stages of HIV-related disease, different degrees of immune damage can be modeled. We chose to examine representative viruses known to be found in saliva, blood, or urine, both because of ease of sample collection from study volunteers and because they raise the possibility of contamination and spread within the spacecraft environment. Herpesvirus EBV replicates in the oropharynx and parotid glands and virus is present in saliva. It also infects and immortalizes B lymphocytes, although at any given time very few of the lymphocytes release virus particles. Polyomaviruses establish infections of the kidneys that may result in viruria; these viruses also may be present at very low levels in PBMCs. DNAs were extracted from PBMCs, urine cell pellets, and saliva and were tested for the presence of viral DNAs by PCR. Selected amplicons were further characterized by sequence analysis.

b. Longitudinal Study of Normal Volunteers: In order to interpret virus reactivation studies in ground-based analogs of space flight, we needed to collect and analyze similar data obtained from normal healthy volunteers. To address whether healthy individuals control virus

reactivation globally or independently and to identify patterns of sporadic reactivation, we monitored herpesviruses and polyomaviruses in 30 healthy individuals over 14 months. EBV DNA was quantitated in saliva and PBMCs, CMV was assayed in urine, and JCV and BKV DNAs in urine and PBMCs. All individuals shed EBV in saliva on at least one occasion, whereas 67% had >1 blood sample positive for EBV. Levels of EBV varied widely. Distinct EBV genotypes were identified and multiple EBV infections were found in 2 of the 9 individuals studied. These results demonstrate that EBV infection with multiple genotypes of healthy individuals is not uncommon and may be a normal aspect of EBV biology. CMV excretion occurred infrequently, but more commonly in younger individuals ($p < 0.03$). JCV and BKV viruria were 46.7% and 0%, respectively. JCV shedding was age-dependent, occurring commonly in persons >40 years ($p < 0.03$). There was no correlation among shedding of EBV, CMV, and JCV ($p > 0.50$). Thus, healthy individuals independently control persistent viruses, which display discordant, sporadic reactivations. (Ling, et al. *J Infect Dis* 187:1571-1580, 2003; Walling, et al. *J Virol* 77:6546-6550, 2003; see **p. 24.**)

c. HIV Infection: Model for Immune Status and Virus Reactivation: HIV-infected individuals represent a population known to be susceptible to complications from reactivation of latent viruses and that can provide insights into levels of immune damage and viral reactivation. A study was designed involving one-time collections of blood, saliva, and urine from 70 HIV-infected individuals and 40 uninfected controls. We also included the final collections from 28 healthy volunteers from our longitudinal study in the latter group. Using a real-time quantitative PCR assay, we demonstrated that significantly more HIV-infected persons receiving HAART than -uninfected volunteers had detectable EBV DNA in blood (57/70, 81% vs. 11/68, 16%; $p = 0.001$) and saliva (55/68, 79% vs. 37/68, 54%; $p = 0.002$). The mean numbers of EBV copies in blood and saliva were also higher in HIV-infected than -uninfected volunteers ($p = 0.001$). The frequency of EBV detection in blood was associated with lower CD4 cell counts ($p = 0.03$) among HIV-infected individuals, although no differences were observed in the EBV DNA loads in blood or saliva within the HIV-infected group. (Ling, et al. *Clin Infect Dis*, in press, 2003; see **p. 24.**)

We also examined humoral responses to EBV and EBV DNA loads in the blood from a cohort of HIV- infected patients in collaboration with Dr. C. O'Sullivan at the University of Alabama, Birmingham. Unlike the HIV study described above, this patient cohort had very low average CD4 counts (under 150/ μ L). Significant increases in the levels of Epstein-Barr nuclear antigen (EBNA) and Epstein-Barr early antigen (EA) antibodies were demonstrated in the 17 patients who had a good response to HAART. Of 29 patients with paired samples tested, four-fold or greater increases in titers were detected for EA in 12/29 (41%), for EBNA in 7/29 (24%), for VCA-IgG in 4/29 (14%); four-fold decreases in titers were detected in 2/29 (7%) for EA and 12/29 (41%) for EBNA. In summary, we demonstrated immune reconstitution to EBV, as measured by EBV serological and DNA levels in patients with advanced HIV infection treated with HAART. The data provide yet more evidence of the remarkable ability of the immune system to recover once HIV replication is markedly reduced by HAART. (O'Sullivan, et al. *J Med Virol* 67:320-326, 2002; see **p. 24.**)

Polyomavirus reactivation and shedding were examined in the HIV study also. JCV excretion in urine was elevated in the HIV-positive cohort (22/70, 31.4%), compared to the HIV-negative group (13/68, 19.1%), but not significantly different ($p = 0.09$). No BKV shedding was detected

and all blood samples were negative for polyomavirus DNA sequences. Strikingly, the age-related pattern of JCV shedding in uninfected subjects ($p=0.01$) was inverted in the HIV-infected individuals, with the younger age groups exhibiting rates of JCV excretion as high as the ≥ 40 age group ($p=0.13$). Among HIV-infected patients, JCV reactivation was more frequent in subjects with lower CD4 cell counts ($p=0.03$). We extended the polyomavirus study by performing genotype (sequence) analysis on the JCV excreted by both groups of subjects. All were JCV archetypal strains. JCV genotypes 1 (36%) and 4 (36%) were the most common among HIV-infected patients, whereas type 2 (77%) was the most frequently detected among HIV-uninfected volunteers. As the common types of JCV excreted varied among ethnic groups, JCV genotypes reportedly associated with progressive multifocal leukoencephalopathy may reflect demographics of those infected patient populations. (Lednicky, et al. AIDS 17:801-807, 2003; see **p. 24.**)

These studies demonstrated the crucial role that host immune function plays in regulation of latent and persistent viral infections. Even modest depressions in immune function correlate with virus reactivation, emphasizing the importance of understanding the effect of long-duration space flight on the host immune system. In addition, we established significant benchmarks that are useful in interpreting viral reactivation data from space flight analog studies.

d. Viral Associations with Non-Hodgkin Lymphomas: Non-Hodgkin lymphoma (NHL) has increased in frequency over the past 30 years and is a common cancer in organ transplant recipients and in HIV-1-infected patients. Although no definite risk factors have emerged, a viral cause has been postulated. Polyomaviruses are known to infect human beings and to induce tumors in laboratory animals, including the production of B-cell lymphomas by SV40. We aimed to identify which one of the three polyomaviruses able to infect human beings (SV40, JCV, BKV) was associated with NHL, as well as the association of EBV. We analyzed systemic NHL from 76 HIV-1-infected and 78 HIV-1-uninfected patients and nonmalignant lymphoid samples from 79 HIV-1-positive and 107 HIV-1-negative patients without tumors; 54 colon and breast carcinoma samples served as cancer controls. Polyomavirus T-antigen sequences, all of which were SV40-specific, were detected in 64 (42%) of 154 NHL by PCR/Southern blot analysis but not in control samples. The detection rate of SV40 DNA was significantly higher in samples of NHL than in nonmalignant lymphoid samples from HIV-1-infected patients (25/76, 33% vs. 0/79, 0%; $p<0.0001$) or HIV-1-uninfected patients (39/78, 50% vs. 0/107, 0%; $p<0.0001$). The rate was also significantly higher in NHL from HIV-1-uninfected patients than in cancer control samples (39/78, 50% vs. 0/54, 0%; $p<0.0001$). NHL samples were more frequently EBV-positive in HIV-1-infected than HIV-1-uninfected patients (30/76, 39% vs. 12/78, 15%; $p=0.001$), whereas NHL samples were more frequently positive for SV40 in HIV-1-uninfected than HIV-1-infected patients (39/78, 50% vs. 25/76, 33%; $p=0.03$). Few tumors were positive for both SV40 and EBV. Human herpesvirus type 8 was not detected. This study established that viruses shown to be reactivated under space flight conditions (herpesvirus EBV, polyomaviruses) are associated with human malignancies (lymphomas). (Vilchez, et al. J Acquir Immune Defic Syndr 29:109-116, 2002; Vilchez, et al. Lancet 359:817-823, 2002; see **p. 24.**)

e. Winter-Over in Antarctica: This study, in collaboration with the Australian Antarctic Division (Dr. D.J. Lugg), started in November 1998 (beginning of Antarctic summer). Experiments were conducted during the Antarctic winter of 1999 (March to October), and specimens were returned in minus 80 degree freezers on ships from the Australian National

Antarctic Research Expeditions (ANARE) in Antarctica to headquarters in Australia (December 1999–February 2000) and flown to Houston in mid-2000 in dry ice. Experimental subjects were stationed at three Antarctic outposts (Mawson, Davis, Casey), and control subjects were stationed on Macquarie Island, 2000 miles from the Antarctic continent. The subjects provided weekly saliva and urine samples and bimonthly plasma and buffy coat (WBCs) specimens, starting 3 weeks before leaving Australia for Antarctica and ending 1 week after returning to Australia. We received >3500 specimens, but we have not yet tested all the specimens because of the expense of the assays. These samples proved invaluable in a publication on alteration of cytokine function and reactivation of latent virus infection, as reported by Shearer, et al., 2002.

The urine samples were analyzed for the presence of polyomaviruses. JCV excretion was detected in samples from 10 of 63 (15.9%) test subjects and BKV was shed by 5 (7.9%) individuals. Two control groups were evaluated to complement data from the healthy volunteer study carried out in Houston. In addition to the subjects on Macquarie Island, we included normal volunteers in Australia, as the Antarctica expeditioners were all Australian. In the US healthy volunteers, JCV shedding was strongly age-dependent, with the highest level (64.7%) of virus shedding by individuals 40 years or older. A similar age-related pattern for JCV excretion was observed among the Australian normal volunteers. It is of interest that the Antarctica expeditioners showed comparable levels of JCV shedding by individuals in the 30.39 year-old age group as in the ≥ 40 year-old group. No polyomaviruses were detected in specimens from the individuals posted on Macquarie Island. This may reflect the difficulties encountered in sample collection, storage, and transport under the less-than-ideal Antarctica expeditionary conditions; the quality of samples returned from the different outposts varied significantly. Although no excretion of BKV was detected in the longitudinal study of US healthy volunteers or in the volunteers stationed at Macquarie Island, BKV was recovered from 6/30 (20.0%) of the Australian control group. Therefore, the detection of BKV in some Antarctica samples may reflect the Australian background of the polar expeditioners. None of the differences between groups was statistically significant.

f. Russian Closed-Chamber Study: Attempts to integrate this NSBRI project with chamber studies at NASA/JSC were thwarted when planned chamber studies were cancelled. As an alternative, we received approval to participate in a 240-day closed chamber study (SFINCSS-99) taking place in Moscow, Russia, by the Institute for Biomedical Problems (Dr. I. Larina, collaborator). The design of the study is shown in **Figure 12**. Four crew members were confined in the chamber for the duration of the study and were visited by two 4-person crews, each for 110 days, modeling future crew interactions in the International Space Station. All crew members provided weekly saliva and urine samples and monthly plasma and buffy coat specimens, starting 4 weeks before chamber entry and ending 4 weeks after chamber exit. We hand-carried reagents and supplies to Moscow before the chamber closed in July 1999. When the study ended, Dr. Larina transported specimens to Houston in Fall 2000. Specimens were analyzed as described above for the normal volunteer and HIV-infection studies. To date, we have completed testing only monthly urine, blood, and saliva samples.

We used real-time PCR to detect EBV in blood and saliva from Crews #1 and #2 and are currently determining EBV levels from Crew #3. All crew members shed EBV in their saliva on multiple occasions. An example of EBV shedding from Crew #1 before, during, and after chamber enclosure is shown in **Figure 13**. The level and frequency of shedding before entry into

the chamber vs. habitation in the chamber did not change appreciably, but the individuals in both Crews (#1 and #2) shed virus with greater frequency than our normal US cohort [e.g., 60/72 occasions (83%) compared to 54%]. Detection of EBV in PBMCs from crew members was similar to that observed in our normal volunteers with an incidence of 15% (unpublished observation). No changes in EBV detection in the blood were observed among pre-chamber entry, chamber habitation, and post-chamber exit. Polyomavirus JCV shedding was detected in 7/12 (58%) crewmembers, with 3 individuals identified as frequent shedders. More detailed interpretation of the data awaits the additional information of individual crewmembers.

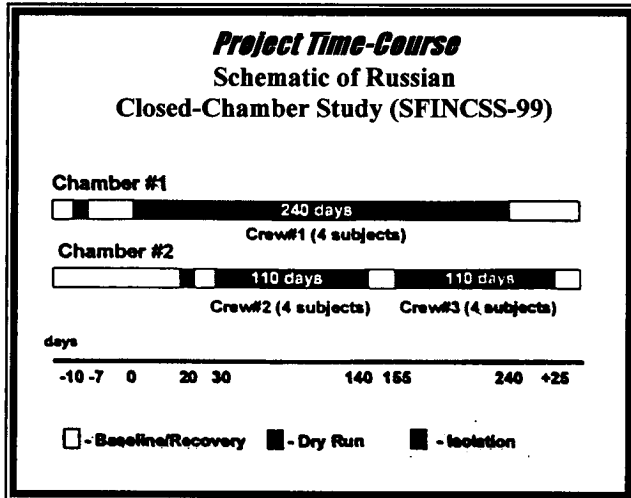


Figure 12

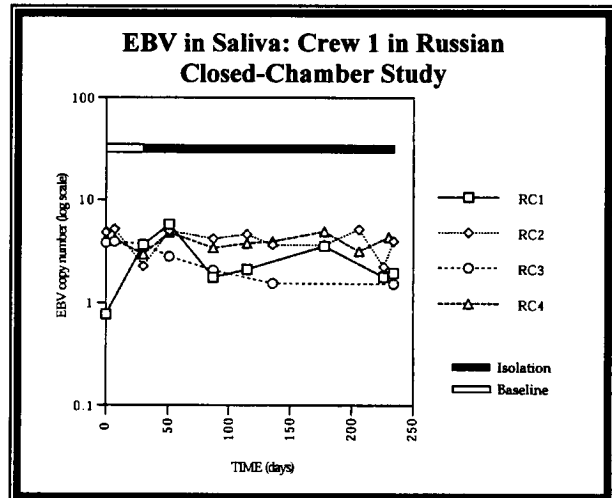


Figure 13

However, all members of Crew #3 were under the age of 40, and we observed that 3 (75%) excreted JCV and 1 (25%) excreted BKV. It is of interest that this crew reportedly experienced psychosocial problems. We plan to complete the analysis of the remaining blood, urine, and saliva samples from this study during the coming year and prepare a manuscript. Immunological and CMV assays remain to be completed. Cortisol levels and cytokines will be measured in plasma samples. This will be the first long-duration chamber isolation experience to yield integrated immunological and virological studies.

2. Determine the Effect of Irradiation on Immune Parameters and Susceptibility to Virus Infection. A major risk to long-duration space missions is chronic exposure to ionizing radiation, which can damage host cells and immune function. The hypothesis being tested is that space radiation will lead to reactivation of viruses, increased viral infections, and the development of virus-associated malignancies. The use of animal models is necessary to evaluate the effects of space flight conditions on the host immune response and resistance to microbial infections. With animal models it is possible to control experimental conditions, to collect samples at desired times, to obtain tissue and organ samples not available from humans, to accumulate adequate numbers for statistical analysis, to study basic mechanisms, to examine life-threatening conditions (e.g., radiation), and to test candidate countermeasures not yet approved for human use. During this funding period, we developed a rodent model using murine polyoma virus (PyV) as a mouse equivalent of human JCV and BKV. These studies are being performed in collaboration with Drs. W.T. Shearer and J.M. Reuben of the NSBRI Immunology, Infection and Hematology Team. We expect the animal studies to generate hypotheses that can be tested in the future in human ground-based models.

a. Effect of Irradiation on Murine Polyoma Virus Replication and Persistence in Mice A pilot experiment with Dr. D.S. Gridley at Loma Linda University revealed that proton irradiation and gamma irradiation had similar effects on the murine immune response, so we are currently using gamma irradiation as that avoids the logistical difficulties of shipping animals following treatment from California to Texas. A subset of 6- to 8-week-old BALB/c mice were whole-body gamma irradiated using a Gammacell Irradiator (caesium-137 source; Atomic Energy of Canada, Ltd.). Mice were inoculated intraperitoneally with different amounts of PyV (25-128 hemagglutinating units; HAU). At different times postinoculation (p.i.), tissues were collected, weighed and DNA extracted. Virus was detected using a specific PCR method (1.2 µg DNA/reaction). Initially, we monitored virus replication in 8 tissues at different time points after infection (days 3, 7, and 20) of normal mice. Peak virus detection occurs 7-10 days p.i. Duration of persistence of virus in different tissues was dependent on the dose of virus inoculated. At high doses, some tissues retained virus at detectable levels for up to 20 days (usually kidney, spleen, salivary gland, and liver), whereas other tissues had cleared the virus by day 20 (usually lung, skin, bone, and mammary gland). We also compared the responses of male and female mice to infection with polyomavirus and viral clearance; no significant differences were found. Having established the parameters of virus infection and replication in normal animals, experiments were initiated to evaluate the effect of gamma irradiation on host control of viral infection. We found that gamma irradiation delayed the clearance of virus infection, with persistence of detectable virus in several tissues (kidney, liver, spleen and salivary gland) through day 20 or longer (unpublished observation).

b. Reactivation of Latent Polyoma Virus Infections by Irradiation: Next we explored whether gamma irradiation can cause reactivation of latent viral infections (Fig. 14). Animals irradiated on day 0 and inoculated with 75 HAU of PyV on day 1 displayed elevated virus replication on days 10 and 20 as compared to nonirradiated mice (based on % PCR-positive samples). Spleen, kidney and salivary gland tissues were assayed from 4 mice per time point. Both irradiated and nonirradiated mice cleared the virus infection to undetectable levels. Some animals were then irradiated on day 49 and at day 59 virus was detectable in the spleen, evidence of virus reactivation and replication.

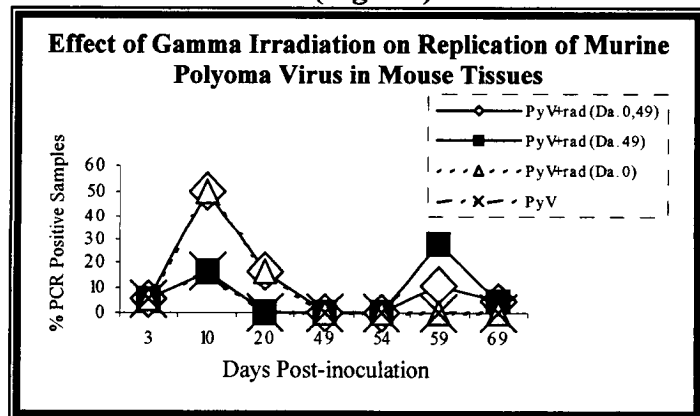


Figure 14

c. Polyoma Virus Strain Differences: Polyomaviruses can differ in the structure of their regulatory region (the noncoding part of the viral genome that controls viral gene expression and DNA replication). Changes especially are found in the enhancer region that binds multiple transcription factors. Viruses with complex enhancer (c.e.) regions replicate faster and to higher titers in tissue culture than those with simple enhancers. We compared the two types of virus in the mouse model. Irradiated groups were exposed to 3 Gy gamma radiation on day 0, were infected with 75 HAU of virus on day 1, and irradiated again on day 30. There were 4 mice per time point; tissues tested were spleen, kidney, salivary gland, and liver. Virus replication was elevated (more detectable) in specimens from the acute phase of infection (first 2 weeks) from

irradiated animals as compared to the nonirradiated, followed by virus clearance by day 20 (Figures 3, 4 above). Interestingly, following a second irradiation on day 30, reactivation was observed only with the virus with a simple enhancer (s.e.). This was the pattern in spleen and kidney, whereas in other tissues (salivary gland, liver), virus was not completely cleared by the irradiated animals. These observations support a model in which a better-replicating virus that produces more virus antigen and presumed cell damage is cleared more effectively by the host whereas a poorly replicating virus can escape immune elimination and establish persistent infections.

d. Effect of Irradiation and Infection on Immune Function: The effects of gamma irradiation and PyV infection on immune function are being studied by T-cell proliferation and cytokine production assays in collaboration with Drs. W.T. Shearer and J.M. Reuben. Compared with non-irradiated mice, Con-A- stimulated spleen cells of irradiated mice produced significantly lower levels of IL-2 ($p=0.000$), IFN- γ ($p=0.000$), TNF- α ($p=0.001$), IL-4 ($p=0.000$), and IL-5 ($p=0.003$) as early as day 3. PyV infection of irradiated mice did not adversely affect immune function up to day 12 p.i.; however, on day 31 p.i. spleen cells of PyV-infected, irradiated mice produced significantly lower levels of IL-2 ($p=0.01$), IFN- γ ($p=0.01$), TNF- α ($p=0.01$), and IL-5 ($p=0.019$) than spleen cells of irradiated mice without PyV infection. The former group of mice produced significantly less Th1 cytokines, IL-2 and IFN- γ than non-irradiated mice that were inoculated with PyV at all time points tested. These preliminary results suggest that a low dose of gamma irradiation of mice leads to immune suppression and increased susceptibility to infection with virus and that the combined effect of irradiation and virus infection further immune suppresses the host. This mouse model will be used for targeted studies of the effects of irradiation on host immune function, virus infection, and tumor development. Such data will help define the potential risk of these combined factors to long-duration space flight and will allow tests of countermeasures.

3. Characterize Changes in Mucosal or Systemic Immune Responses under Simulated Space Flight Conditions with or without Concomitant Virus Infection The mouse anti-orthostatic hind limb unloading (HLU) model mimics some of the changes observed in space flight conditions and is the most relevant ground-based animal model for space flight conditions. However, none of the previous studies on the effects of HLU on infections examined mucosal immune responses or changes in antibodies. Using this mouse HLU model, our goals were to determine whether there are immune system changes in mucosal lymphoid sites for levels of gene expression, cytokine production, or cell distributions.

To model the effects of space flight conditions on clearance of primary infections or protection from rotavirus challenge, CD-1 outbred mice were subjected to HLU for either 4 or 14 days prior to rotavirus infection or challenge. Clearance and protection from rotavirus require mucosal immune responses. Except for a minor change in IgG subclass, no differences in protection or antibody responses were observed between rotavirus-infected HLU or control mice. HLU causes changes in innate immune responses (IFN- α and macrophages). Therefore, our results suggest that these innate immune responses are not critical to protection against rotavirus, which fits well with current data in the rotavirus field. The limited changes induced by HLU on serum and mucosal antibody responses, the transient changes in B cell numbers induced by irradiation of mice, and the normal antibody responses in Antarctic expeditioners suggest that space flight conditions may minimally affect antibody responses. Therefore, countermeasures targeting

induction of antibody responses may be feasible. This work has been submitted for publication (Blutt and Conner).

One of our goals was to identify changes in the mucosal and systemic immune system caused by hind limb unloading, independent of the effects of a concomitant infection with a pathogen or irradiation. We hypothesized that hind limb unloading alone would induce a stress response resulting in immunosuppression. We examined whether changes in *basal* levels of cell distribution occur and whether altering the length of hind limb unloading temporally regulates these changes. We compared results from CD-1 female mice housed under three conditions, normal (singly-housed), restrained (orthostatic), or hind limb unloaded at 0, 3, 7, and 12 days post suspension (dps). Five mice from each group were harvested at each time point. We compared changes at both systemic (spleen) and mucosal (Peyer's patches and mesenteric lymph node) sites. Tissues were collected, single cell suspensions made, and total numbers and percentage of different populations of cells were examined by flow cytometry. We examined distribution of cells by quantitating the number of B cells (CD19), subsets of T cells (CD4 and CD8), and macrophages (Mac3) at different sites. Significant differences were not observed between hind limb suspended and restrained mice, with the exception of an increase in the percentage of CD8+ T cells in the mesenteric lymph node at 7 dps. However, significant differences were noted in the total numbers or percentages of different cell populations in both

hind limb unloaded and restrained (orthostatic) mice compared to normally housed mice (**Table 3**).

Virtually all the changes noted were early, at 3 or 7 dps. The kinetics of these differences varied in the different tissues; changes were noted in the Peyer's patches at 3 and 7 dps, mesenteric lymph node at 7 and 12 dps, and in the spleen at 3 dps. Interestingly in Peyer's patches and mesenteric lymph nodes, the majority of the changes noted were decreases (B, CD4+, CD8+, macrophages), while in the spleen there were increased CD4+ and CD8+ cells but decreased B cells. These results appear to support our hypothesis that stress-related changes in lymphocyte distribution occur for limited duration at mucosal and systemic sites both due to hind limb unloading and restraint (orthostatic) and that these changes differ at different lymphoid sites. These

Table 3. Changes in leukocyte and monocyte distribution following hind limb suspension or restraint (orthostatic) of mice.

Site	Days post suspension		
	3	7	12
PP	CD4% dec	CD8% inc	No changes
	Total cells dec	Total Cells dec	
	Total CD19 dec	total CD4 dec	
	total Mac3 dec	Total Mac3 dec	
MLN	No changes	Total cells dec total CD4 dec Total CD8 dec	Mac % inc
	Spleen	No changes	No changes
		CD4% inc CD8% inc CD19 % dec	
Orthostatic relative to control also changed (same direction)			

results fit with the limited data available from space flights that indicate that there is limited, but not profound, immunosuppression and that the observed changes are often more pronounced during highly stressful portions of the flight such as takeoff and landing.

Once the PyV model was established, we focused our efforts there to examine possible immune system changes. We have now established low density membrane based gene arrays (SuperArray Inc.) in the laboratory to examine changes in the immune response. We have stored frozen tissues from the PyV-infected and irradiated mice for gene expression analyses of changes in cytokine gene expression. We had proposed to assess hind limb unloading effects on cytokine proteins. This will now be done on hind limb unloading control groups in the PyV project, thereby limiting the total number of mice needed for our studies.

Publications:

- **Blutt SE, Conner ME.** kinetics of rotavirus infection in mice are not altered in a ground-based model of space flight. *Aviat Space Environ Med*, in press, 2003.
- **Lednicky JA, Halvorson SJ, Butel JS.** PCR detection and DNA sequence analysis of the regulatory region of lymphotropic papovavirus in peripheral blood mononuclear cells of an immunocompromised rhesus macaque. *J Clin Microbiol* 40:1056-159, 2002.
- **Lednicky JA, Vilchez RA, Keitel WA, Visnegarwala F, White ZS, Kozinetz CA, Lewis DE, Butel JS.** Polyomavirus JCV excretion and genotype analysis in HIV-infected patients receiving highly active antiretroviral therapy. *AIDS* 17:801-807, 2003.
- **Ling PD, Lednicky JA, Keitel WA, Poston DG, White ZS, Peng RS, Liu Z, Mehta SK, Pierson DL, Rooney CM, Vilchez RA, Smith EO, Butel JS.** The dynamics of herpesvirus and polyomavirus reactivation and shedding in healthy adults: a 14-month longitudinal study. *J Infect Dis* 187:1571-1580, 2003.
- **Ling PD, Vilchez RA, Keitel WA, Poston DA, Peng RS, White ZS, Visnegarwala, F, Kozinetz CA, Lewis D, Butel JS.** Epstein-Barr virus DNA loads in adult HIV-infected patients receiving highly active antiretroviral therapy. *Clin Infect Dis*, in press, 2003.
- **O'Sullivan CE, Peng RS, Cole KS, Montelaro RC, Sturgeon T, Jenson HB, Ling PD.** Epstein-Barr virus and human immunodeficiency virus serological responses and viral burdens in HIV- infected patients treated with HAART. *J Med Virol* 67:320-326, 2002.
- **Vilchez RA, Lednicky JA, Halvorson SJ, White ZS, Kozinetz CA, Butel JS.** Detection of polyomavirus simian virus 40 tumor antigen DNA in AIDS-related systemic non-Hodgkin lymphoma. *J Acquir Immune Defic Syndr.* 29:109-116, 2002.
- **Vilchez RA, Madden CR, Kozinetz CA, Halvorson SJ, White ZS, Jorgensen JL, Finch CJ, Butel JS.** Association between simian virus 40 and non-Hodgkin lymphoma. *Lancet* 359:817-823, 2002.
- **Walling DM, Brown AL, Etienne W, Keitel WA, Ling PD.** Multiple Epstein-Barr virus infections in healthy individuals. *J Virol* 77:6546.6550, 2003.
- **Sonnenfeld G, Butel JS, Shearer WT.** Effects of the space flight environment on the immune system. *Rev Environ Health* 18:1-17, 2003.

Presentations: Abstracts/Lectures

- **Butel JS, Lednicky JA, Vilchez RA, Visnegarwala F, Lewis DE, Kozinetz CA, and Keitel WA.** Polyomavirus JCV reactivation and shedding in healthy and immunocompromised hosts: implications for space travel. *Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.*

- **Ling PD**, Vilchez RA, Keitel WA, Rooney CM, Smith EO, **Butel JS**. The dynamics of herpesvirus Epstein-Barr virus reactivation and shedding in healthy adults: implications for space travel. Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.
- **Butel JS**, Zhang S, Reuben J, **Shearer WT**. The effect of irradiation on murine polyoma virus replication and persistence in mice: a model for space flight. Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.
- Reuben JM, **Butel JS**, Lee BN, Zhang S, Shen DY, Shearer WT. The effect of gamma irradiation on the immune responses of mice with polyoma virus infection. Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.
- Reuben JM, **Butel JS**, Lee BN, Zhang S, Shen DY, Shearer WT. The effect of gamma irradiation on the immune responses of mice with polyoma virus infection. 14th Space Radiation Health Investigators' Workshop, League City, TX, April 27-30, 2003.
- **Ling P**, Lednicky JA, Keitel W, White ZS, Visnegarwala F, Vilchez RA, **Butel JS**. The shedding of JCV and EBV in HIV-infected patients receiving HAART. 9th Conference on Retroviruses and Opportunistic Infections, February 24-28, 2002, Seattle, WA.

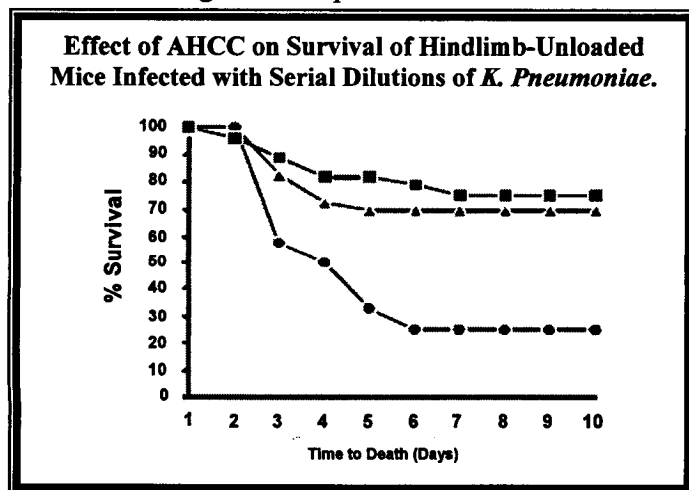
Project 3: Gerald Sonnenfeld, Ph.D., Principal Investigator; "Suspension, the HPA Axis, and Resistance to Infection" (addresses Strategic Plan Risks 2-5 and Critical Questions 7.03, 7.06, 7.11)

The current application is designed as an extension of our previous findings funded by a NSBRI grant entitled "Suspension, the HPA Axis and Resistance to Infection". The specific aims of that grant were: 1) to expand the range of infections altered by HU. We had previously shown that resistance to some infections that are not likely to be risks during space flight had been altered by HU. In the NSBRI grant, we designed studies to determine if resistance to infections that could be a risk during space could be altered by HU; and 2) to determine the mechanism of alteration of resistance to infection induced by HU. Although previous studies have shown that immune responses are altered by space flight, we extended those studies to determine the role of neuroendocrine system in regulating infections. This was carried out using two approaches. The first approach involved an evaluation of HU-induced alterations of neuroendocrine (the sympathetic nervous system and the hypothalamic-pituitary-adrenal [HPA] axis) responses. The second approach involved an evaluation of direct effects of the host neuroendocrine system response on the pathogen.

The results of the studies in the NSBRI grant have been as follows. We carried out a study to determine the effects of HU on resistance of mice to infection with *Klebsiella pneumoniae*. We have shown that mortality was 50% for control mice that were normally caged and housed and received 1 LD50 (lethal dose for 50% of the animals) of *K. pneumoniae*. Mortality was 43% for control mice that were restrained and received 1 LD50 of *K. pneumoniae*. Mortality was 86% for experimental mice that were hindlimb-unloaded and received 1 LD50 of *K. pneumoniae*. Therefore, it appears that HU, which mocks some aspects of space flight conditions, increases mortality to pathogens that could be encountered during space flight. We have also completed studies to determine the effects of hindlimb unloading on resistance of mice to *Pseudomonas*

aeruginosa, a gram-negative bacteria that has created infectious problems during space flight. We have found that suspended mice had significantly enhanced mortality when infected with the bacteria in a fashion similar to that obtained with *K. pneumoniae*. We also demonstrated that the hindlimb-unloaded mice that were infected had a prolonged elevation of corticosterone, indicating that a stress response might play some role in decreasing resistance to infection.

We have begun studies with active Hexose correlated compound (AHCC). AHCC is an extract prepared from cocultured mycelia of several species of Basidiomycete mushrooms. It contains polysaccharides, amino acids, and minerals and is orally bioavailable. It has been shown to have a beneficial effect on the immune system of normal humans and rodents, including enhancement of natural killer cell activity. AHCC is available “over-the-counter” as a nutritional supplement. We were asked by the Amino Up Chemical Company of Japan, the producer of AHCC to test the ability of AHCC to protect mice from infection in the HU model. They made this request based on the results obtained on infection and the HU model in our earlier publications. In the study we carried out with AHCC, mice pre-treated with AHCC and then hindlimb-unloaded were significantly protected from infection with *Klebsiella pneumoniae* (Fig. 15). In fact, protection against infection was greater in HU mice than in normally caged mice (92). This surprising result stimulated us to continue additional mechanistic studies with AHCC. We were able to show that hindlimb-unloaded mice infected with *K. pneumoniae* could not clear the infection from the blood, but hindlimb-unloaded mice pre-treated with AHCC and infected with *K. pneumoniae* were able to clear the bacteria from the blood. These results suggested that the immune system was stimulated by pre-treatment with AHCC, resulting in clearing of the infection. These results allow us to begin development of a countermeasure for immune dysfunction, AHCC, starting at countermeasure readiness level 4.



Data were expressed as percent survival at each time point determined by the Kaplan-Meier method. Difference in survival between the three experimental groups of mice was compared using the Logrank (Mantel-Cox) method ($p < 0.01$). (●) Hindlimb-unloaded mice receiving phosphate buffered saline (PBS); (■) Hindlimb-unloaded mice receiving AHCC; (▲) Normally caged mice not subjected to gavage. All mice were infected. Survival of hindlimb-unloaded mice receiving AHCC was significantly different from hindlimb unloaded mice receiving PBS.

Figure 15

We have also completed studies to determine the effects of catecholamines on the growth of potentially pathogenic bacteria that would be encountered during space flight. Supplementation of minimal medium inoculated with bacteria cultures with norepinephrine, epinephrine, dopamine, or isoproterenol resulted in marked increases in growth compared to controls. Norepinephrine and dopamine had the greatest enhancing effects on growth of cultures of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while epinephrine and isoproterenol also enhanced growth to a lesser extent. The growth of *Escherichia coli* in the presence of norepinephrine was greater than growth in the presence of the three other neurochemicals used in

the study. Growth of *Staphylococcus aureus* was only slightly enhanced in the presence of norepinephrine, but not to the same degree as was the growth of gram negative bacteria. Addition of culture supernatants from *E. coli* cultures that had been grown in the presence of norepinephrine was able to enhance the growth of *K. pneumoniae*. Addition of the culture supernatant fluid culture from *E. coli* cultures that had been grown in the presence of norepinephrine did not enhance growth of *P. aeruginosa* or *S. aureus*. Culture supernatant fluids from bacteria other than *E. coli* grown in the presence of norepinephrine were not able to enhance the growth of any bacteria tested. The results suggest that catecholamines can enhance growth of pathogenic bacteria, which may contribute to development of pathogenesis; however, there is no uniform effect of catecholamines on bacterial growth. Therefore, catecholamines could play a role in increasing growth and expression of virulence factors for some pathogens that could be encountered during space flight. We have completed additional studies to determine the effects of catecholamines on the growth of potentially pathogenic bacteria that would be encountered during space flight. Supplementation of minimal medium inoculated with bacterial cultures with norepinephrine, epinephrine, dopamine, or isoproterenol resulted in marked increases in growth compared to controls. Norepinephrine and dopamine had the greatest enhancing effects on growth of cultures of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while epinephrine and isoproterenol also enhanced growth to a lesser extent. We have now been able to show that the greatest effects of the catecholamines are not on gram-positive organisms, i.e. only gram-negative organisms are affected. The enhancing effects of catecholamines appear to be very specific for certain gram-negative bacteria, and anaerobic bacterial growth appears to be unaffected by catecholamine treatment.

We also carried out research with other NSBRI-funded scientists. We have begun studies with Dr. Butel determining the effects of catecholamines on viral growth. These experiments are in progress. Preliminary results indicate that doses of catecholamines that can be used are limited, because high doses of catecholamines can be toxic to mammalian cells required for viral growth. We expect to complete these experiments in the next year. We have also begun studies with Drs. Fox and Willson regarding the use of array analysis to determine exactly what genes are expressed when catecholamines enhance bacterial growth and virulence. These experiments are in progress. We have begun experiment with Dr. Marcello Vazquez at the Brookhaven National Laboratory determine if proton and HZE radiation exposure of mice affects the immune response of those mice. These experiments are in progress. We have just begun studies with Dr. Ann Kennedy of the University of Pennsylvania to determine the effects of with the Bowman-Birk inhibitor, which is a soybean extract that has been shown to have preventative effects on the development of colon cancer. Our studies, which are currently in progress, involve the determination of the effects of the Bowman-Birk inhibitor on the immune system of hindlimb-unloaded mice. We hope to complete these studies in the next year in time for completion of our current NSBRI grant.

We have been able to show that production of catecholamines correlates with immunosuppression in a model of space flight conditions. The hindlimb-unloading mouse model has been used successfully to simulate some of the physiological effects of space flight. Previous studies in our laboratory have shown that exposure of mice to hindlimb-unloading two days prior to infection is sufficient to induce physiological and immunological changes resulting in decreased resistance to infection. In an attempt to delineate the mechanisms involved, levels of catecholamines were measured in plasma samples obtained from hindlimb-unloaded and

control mice during the suspension period. At the same time, immunological function (spleen and peritoneal cell function) was assessed. Results showed that the levels of adrenaline and noradrenaline were increased in hindlimb-unloaded and restrained mice compared with control normally housed mice. Spleen cell proliferation and peritoneal cell function assessed by nitric oxide production was impaired in the hindlimb-unloaded group compared to both other groups. These results suggest that catecholamines may contribute to the immune dysfunction observed after hindlimb unloading.

We have been able to show that growth of bacteria in the presence of catecholamines alters the production of virulence factors. *Pseudomonas aeruginosa* is an opportunistic pathogen that has been shown to cause infections in immunosuppressed individuals. Infection with *P. aeruginosa* has occurred in astronauts during space flight. Catecholamines, norepinephrine (NE) in particular, enhance the growth and increases expression of virulence factors of bacteria cultured in minimal SAPI medium supplemented with 30% serum. The objective of this study was to investigate whether NE influences the production of exotoxin A (EA) by *P. aeruginosa* cultured in SAPI medium supplemented with 30% serum. About a 100 colonies of *P. aeruginosa* were inoculated into 10 ml of serum-supplemented SAPI medium, Tryptic Soy broth (TSB), simpler defined medium1 (SDM1), and modified simpler defined medium 2 (MSDM2) with or without a 100 µl of 10⁻⁴ M NE for 72 h. Samples were obtained at 24, 48 and 72 h for EA level determination by ELISA and for colony counts. The level of EA production was not detected in the serum-supplemented SAPI medium containing NE despite 2-3 log enhanced growth compared to growth in control cultures. Decreased EA production was observed in TSB, SDM1 and MSDM2 cultures supplemented with serum only. The mechanism of the effects of NE on EA production remains to be established, as does the biological significance of the decreased EA production.

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- Powell JM, Aviles H, Belay T, **Sonnenfeld G**. The effects of dehydroepiandrosterone (DHEA) on *in vitro* spleen cell proliferation and cytokine production. Submitted for publication, 2003.

Presentations: Abstracts/Lectures

- **Sonnenfeld G**. Plenary Speech: Space flight, the immune system and resistance to infection. 11th International AHCC Research Symposium, Sapporo, Japan, July 2003.
- **Sonnenfeld G**. Space flight, the immune system and resistance to infection. Women's Information Network Group Scientific Symposium, Tokyo, Japan, September 2003.
- **Sonnenfeld G**. Space flight, the immune system and resistance to infection. Women's Information Network Group Scientific Symposium, Osaka, Japan, September, 2003.

- **Sonnenfeld G.** Space flight, the immune system and resistance to infection. Women's Information Network Group Scientific Symposium, Nagoya, Japan, September 2003.
- Aviles H, Belay T, Vance M, **Sonnenfeld G.** Increased levels of catecholamines correlate with decreased function of the immune system in the hindlimb-unloading rodent model of space flight (Abstract 107). American Society for Gravitational and Space Biology Annual Meeting, Huntsville, AL, November 2003.
- Belay T, Aviles H, Pyle B, **Sonnenfeld G.** decreased production of exotoxin a during enhanced growth of *Pseudomonas aeruginosa* in serum-sapi medium supplemented with norepinephrine (Abstract 57). American Society for Gravitational and Space Biology Annual Meeting, Huntsville, AL, November 2003.

Project 4: Alan M. Gewirtz, M.D., Principal Investigator; "Human Hematopoietic Pleuripotent Stem Cells" (addresses Strategic Plan Risks 1, 3, 4 and Critical Questions 7.03, 7.04, 7.23, 7.24)

1. Human Hematopoietic Cell Biology

a. Isolation, Characterization, and Assay of Human Hematopoietic Stem (HSC) and Progenitor Cells (HPC): We have a considerable amount of experience with methods employed for the isolation, characterization, and culture of primary human hematopoietic progenitor and "stem" cells. For the sake of brevity, we refer to this experience, and our knowledge of the

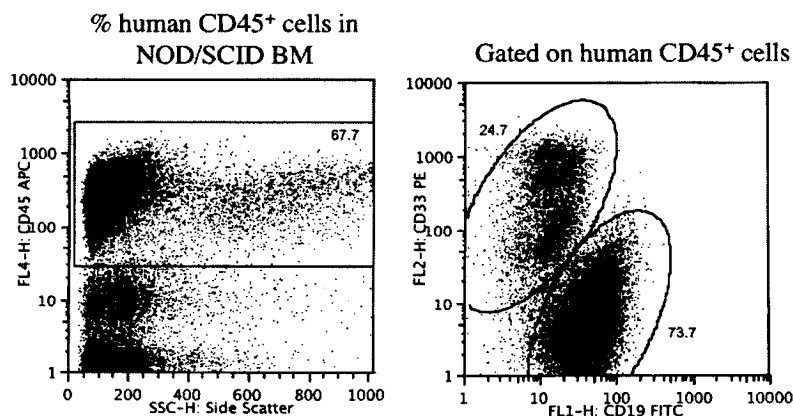


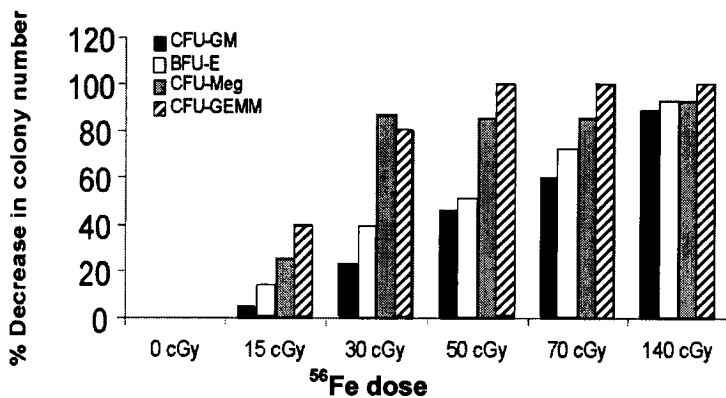
Figure 16 (left): Engraftment of normal human CD34+ cord blood cells in NOD/SCID mouse.

required methodology by citing our own work in this area. The "gold standard" for stem cell identification is the ability of cells to reconstitute hematopoiesis in a live animal. Our animal models have undergone considerable improvement since the inception of this project. We now employ a NOD/SCID/ β 2 model system. Engraftment of primary cells is more efficient in this mouse strain because of a profound defect in NK cell function, in addition to defective T and B cell function.

Cells from normal or human leukemia patients will engraft in NOD/SCID/ β 2 microglobulin knockout animals after they have been irradiated with 250 cGy γ irradiation and then injected with unfractionated mononuclear cells from the donor. For example, when normal cord blood cells ($\sim 1 \times 10^5$) are transplanted into these animals, we now routinely obtain $>50\%$ engraftment. **Figure 16** (above) gives an example of 67% human cell engraftment when the animal was sacrificed ~ 7 weeks post transplantation. Accordingly, these animals provide an excellent model for testing the molecules we develop for ability to protect normal human hematopoietic cells.

b. Cell Biology – Concise Summary of Progress To Date: With studies that initiated in the first phase of this project, we established a dose-response to study the effects of low dose γ -radiation on human hematopoietic progenitor cells. CD34+ cells derived from human bone marrow of normal donors aged between 25-45 years, were exposed to varying doses of γ -irradiation ranging from 15 cGy to 140 cGy. These cells, which include HSC and HPC, were then analyzed for functional consequences of such exposure by examining their ability to proliferate and differentiate in vitro, using the standardized assay of Colony Forming Units (CFU), and long-term culture (LTCIC). We observed a direct correlation between the dose intensity and growth/proliferation of these progenitor cells.

In the second phase, we irradiated human CD 34+ cells using two types of irradiation: 1) Low linear energy transfer (LET) radiation in the form of the ^{137}Cs - γ rays, and 2) High LET radiation in the form of ^{56}Fe ions generated by the Alternating Gradient Synchrotron (AGS) at the Brookhaven National Laboratory (BNL). The effects of the ^{56}Fe particles are of particular concern as they are a major component of galactic cosmic rays found in outer space, and for which shielding is impossible.



We observed that increasing doses of both types of radiation caused increasingly greater decreases in colony number for CFU-GM, BFU-E, CFU-GEMM and CFU-Meg, as shown below in **Figure 17**, and **Table 4**. The decreases observed were more dramatic for ^{56}Fe ions when compared to the ^{137}Cs . This is in accordance with the ‘intensity’ differences in energy that exists between these two kinds of radiation.

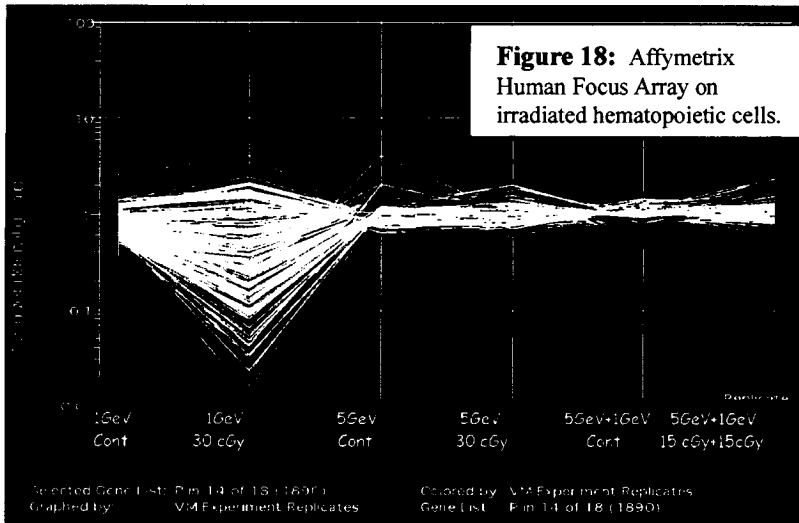
Figure 17 – BNL-8: Response of human HPC to ^{56}Fe , in doses ranging from 0-140 cGy. Each colored bar shows a different CFU assay. Black = granulocyte /macrophage. White = Erythroid. Gray = Megas. Striped = All lineages. Increasing doses decreases the numbers of colonies formed, suggesting that irradiated cells lose the ability to differentiate.

Table 4. Comparison of the Effects of ^{56}Fe and ^{137}Cs Irradiation Using Percent Decrease Compared to Unirradiated Control

cGy	CFU-GM*		BFU-E*		CFU-GEMM*		CFU-Meg*	
	^{56}Fe	^{137}Cs	^{56}Fe	^{137}Cs	^{56}Fe	^{137}Cs	^{56}Fe	^{137}Cs
0	0	0	0	0	0	0	0	0
15	4	19	13	24	40	33	25	25
30	22	24	39	35	80	51	87	55
50	45	37	50	48	100	62	86	72
70	59	45	72	65	100	75	86	88
140	89	67	93	84	100	93	93	98

* Numbers shown are the percent decrease in colonies formed compared to the unirradiated control.

These results indicate that low doses of both ^{56}Fe and γ -radiation can greatly affect the proliferative capacity of hematopoietic progenitor cells. This was further confirmed using the LTCIC assay wherein ^{137}Cs -irradiated human CD34+ cells were cultured on a layer of stroma for 4-5 weeks following which they were assayed for the formation of mature colonies in methylcellulose. This assay was done using limiting dilution analysis. The data obtained showed that a 140 cGy irradiation dose resulted in a 84% decline in LTCIC formation when compared to the unirradiated control.



During BNL-9 we attempted to examine the effect of multiple doses of various particles including Fe, and Si. Unfortunately an incubator accident led to loss of cultures and no data were obtained. We were successful however in generating microarray data on some of these samples, using the Affymetrix Human Focus Array. These data are presented in raw form from duplicate arrays in **Figure 18** (left). For each treatment condition, experimental signal

was compared to its own control because of differences in cell culture time before harvesting. At 5 GeV, 18 of the 27 genes that decreased significantly also decreased significantly following a 1 GeV exposure. Of interest among these were several RNA processing enzymes, including Dicer, the enzyme responsible for processing dsRNA into RNAi inducing short double strands. This may represent a novel, and unanticipated mechanistic component of cellular response to radiation induced damage.

2. DNA Damage Induction and Quantitation

a. Basic Principles: We have developed highly sensitive methods for quantifying DNA damages at low frequencies in nanogram quantities of non-radioactive genomic DNA. The current limiting sensitivity using this method, for damages affecting one strand, is ~ 1 damage site/5 million bases, *i.e.*, 0.2 sites/Mb. For damages affecting both strands (e.g. double strand breaks, clustered damages), the current sensitivity is ~ 5 sites/ 10^9 bp, or 5 sites/Gpb. Clustered damages amenable to quantitation in this system include multiple, closely spaced lesions on opposing DNA strands, for example, on one strand an abasic site, frank single strand break or oxidized purine, and on the opposite strand, an abasic site or an oxidized purine (Note that two closely staggered single strand breaks constitute a double strand break). Such complex sites containing at least one oxidized purine can be quantitated by subdivision of DNA (isolated and NotI digested), and treatment with Fpg protein (while the companion sample is incubated without further treatment) and electrophoresed using neutral gels and a suitable electrophoretic regime. Similarly, complex sites involving oxidized pyrimidines can be measured using Nth protein, and clusters involving abasic sites by using an apurinic endonuclease without associated glycosylase activity such as *E. coli* Nfo protein.

Clustered DNA damages (two or more lesions, including oxidized purine, oxidized pyrimidines, abasic sites and single strand breaks, located within about 10-20 bp of each other on opposing strands) are hypothesized to be critical, repair-resistant lesions producing the harmful effects of ionizing radiation (Similar clusters have also been termed locally multiply damaged sites).

b. Radiation Damage – Concise Summary of Progress to Date: Two critical questions are (1) whether clustered damages are formed in human cells at the low doses (due to the low dose rate) of radiation anticipated during space travel, and (2) whether human primary progenitor and stem cells can repair such clusters effectively. In addition, practical problems we faced were (a) isolation and growth methods for the primary cells that gave preparations suitable for cluster analysis, and (b) identifying the best shipping and transportation methods for getting the cells from U.Penn to BNL (and return, for Dr. Gewirtz's portions of the project.) After resolving the two practical problems, we tackled the critical questions:

3. Are Clusters detectable in DNA of human cells exposed to low doses of space radiation? If clusters—which by definition contain at least two individual DNA lesions—were formed by two independent radiation “hits,” they would not be detectable at the low dose rates experienced during space travel. However, if a heavy charged particle traversing a cell produces

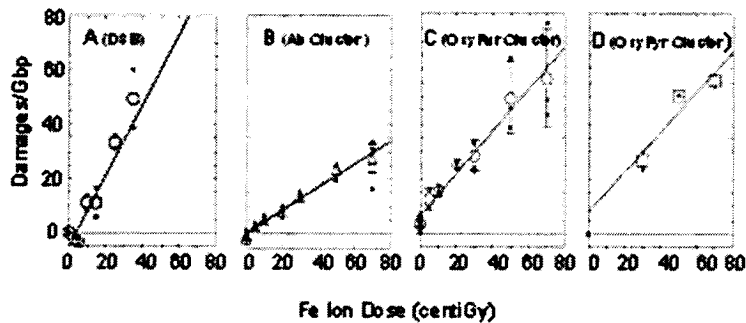


Figure 19. DSB (A), Abasic Clusters (B), OxyPurine Clusters (C), and OxyPyrimidine Clusters (D) induced in human monocytes by 1 GeV/amu Fe Ions. Small solid points, individual data points; open symbols, averages, Error bars, SD.

many ionized species, including water, they could induce many closely spaced DNA lesions, thus producing a clustered damage even at low radiation doses. To distinguish these possibilities, we determined the absolute levels (clusters/ Gigabase pair; Gbp = 10^9 base pairs) induced in human monocytes by Fe ions (1 GeV/amu from Brookhaven's NASA-sponsored Radiobiology Runs at the BNL AGS). **Figure 19** (left) shows our new data for

clustered damages induced by Fe ions corresponding to the four complex damage classes shown above. For these cells, the doses correspond to one to a few hits per nucleus (1 Fe ion “hit” per nucleus at ~17 cGy). Clearly, clusters are formed at low doses, and the dose-response relations are straight lines. These results, as well as our low LET radiation data indicate that clusters are formed by one radiation “hit” (including its accompanying track of ions), and thus pose a potential hazard for humans during space travel.

In comparison with our data for cluster induction by 50 kVp X-rays, the new Fe data show that, in cells exposed to high LET radiation, double strand breaks comprise more of the measurable complex damages relative to OxyBase and Abasic Clusters. However, it is not clear whether fewer such Clusters are actually induced by high LET space radiation, or whether Clusters are induced but are not detected in our standard approach because they contain so many closely spaced lesions that the enzymes we use to recognize clusters cannot cleave. To test these

possibilities, we devised and validated a new approach for cleaving DNA at Abasic clusters refractory to standard Nfo protein cleavage. We are now using this method to determine whether high LET radiation actually produces such high complexity clusters. These data should allow more complete determination of the levels and complexities of DNA damages induced by space radiation.

4. **Can Primary Human Progenitor and Stem Cells Repair Clustered Damages Effectively?**

Clustered damages were held to be of high biological significance because of their potential difficulty of repair. As a basis of comparison for studies of repair of clusters induced by high LET particles, we measured repair of double strand breaks, abasic clusters and oxidized

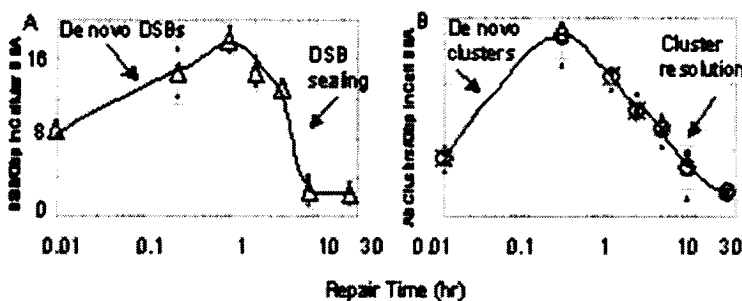


Figure 20. DSBs and Abasic Clusters in primary human hematopoietic cells as a function of incubation time at 37° after exposure to ~1 hit/cell nucleus (20 cGy) of Fe ions. Solid points, individual data points; open symbols, averages. Error bars, SD.

base clusters induced by X-rays (Sutherland et al., submitted; Georgakilis et al., submitted). Since the heavy ion beam was available only once per year, and the primary hematopoietic cells are available on a limited schedule, we used human monocytes for developing methods and obtaining baseline data. These studies showed first, that in human monocytes, double strand breaks disappeared rapidly, and few additional double strand breaks (hypothesized to be generated during cluster repair) could be

detected. Second, abasic clusters were highly persistent, and significant levels of additional abasic clusters—presumably generated as repair intermediates—were observed. Finally, oxidized base clusters were apparently quite repair-refractory, being detectable for many hours after irradiation. Using these approaches, we have now measured repair of clusters induced in human primary hematopoietic cells by Fe ions (1 GeV/amu). **Figure 20** shows the data for processing of clustered damages by the primary hematopoietic cells.

In the first volunteer, resealing of double strand breaks remained rapid; some additional de novo DSBs—presumably generated during attempted repair of clusters, thus producing simultaneous scission of both DNA strands—were seen, but were soon not detectable, presumably resealed. Repair of Fe ion-induced abasic clusters was slower than observed for low LET radiation. As soon as additional heavy ion beam time can be obtained (July, 2003), we plan to obtain independent repeats of these data in primary hematopoietic progenitor and stem cells from additional donors to ascertain their generality. These studies should establish both the specific repair strategy used by this type of human cells, as well as the range of responses in cells from individual donors.

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Presentations: Abstracts/Lectures

- Stanislaus M, Bennett P, Guidal P, Danet G, Luongo J, **Sutherland B**, **Gewirtz A**. Oral Presentation: Effect of deep space radiation on human hematopoietic stem cell function. 31st Annual Meeting of the International Society for Experimental Hematology, Montreal, Canada, July 2002.
- Miller V, Kalota A, Bennet P, Millholland J, **Sutherland B**, **Gewirtz AM**. Platform Talk: Effect of $^{56}\text{Fe}^{26+}$, and Si on human hematopoietic progenitor cell function. 14th IAA Humans in Space Symposium, Banff, Alberta, Canada, May 2003.
- **Sutherland BM**, Bennett P, Georgakilas A, Hada M. Bistranded DNA damage clusters induced by low let radiation and heavy charged particles: formation and repair. NASA Space Radiation Health Investigators' Workshop, Houston, TX, May 2003.

Project 5: Yufang Shi, D.V.M., Ph.D., Principal Investigator; “Endogenous Opioid-Mediated Fas Expression in Stress-Induced Apoptosis” (*addresses Strategic Plan Risks 1, 2, 4 and Critical Questions 7.03, 7.04, 7.23, 7.24*)

Background: Our lab has been investigating the role of activation-induced apoptosis in the regulation of the immune system for several years. Based on our concept of activation-induced cell death (AICD), we have focused on TCR regulated expression of TNF family members and their receptors. We have demonstrated that opioids upregulate Fas expression and that restraint stress-induced lymphocyte reduction is mediated by endogenous opioid-induced Fas expression. Recently, we have found that differential expression of and sensitivity to both FasL and TRAIL in Th1 and Th2 cells, and blocking of apoptosis during T helper cell differentiation leads to an enhanced Th1 response. Our past and current studies have provided the necessary background to investigate how apoptosis modulates the immune system during space flight, especially the role

of Fas and endogenous opioids. In the last 14 months, since funding of this project by NSBRI began on April 1, 2002, we have made significant progress in our projects. We have established our ground-based experimental system, HU in mice, and have obtained some interesting results with this model. Some of these results are described in our recent publications and others will be submitted soon. These results provide the basis for the studies proposed herein.

Hindlimb Unloading Causes Severe Depletion of Lymphocytes in the Spleen and Thymus

We have reported that mice subjected to restraint stress show significant reductions in splenocyte numbers. We have now observed a similar loss of cellularity in mice after HU, a ground-based animal model simulating space flight conditions. In these experiments, mice are suspended by the tail traction (body axis and cage floor form a 25 to 30 degree angle) so that only the forelegs contact the floor. Controls are housed under identical conditions, with the tail restrained, but not suspended. Mice from each group were sacrificed at the indicated times, and cell numbers and phenotype in the spleen and thymus were determined. After 2 days of HU T cells in the spleen were reduced by 45%, while CD19+ B cells were less slightly less affected (Table 5). By day 4, fully 50% of splenic T cells and B cells were lost. In the thymus, immature CD4+CD8+ thymocytes were the most sensitive, dropping by 70% after only 2 days. The total number of mature single positive (CD4+CD8- or CD4-CD8+) thymocytes was also reduced, but to a lesser degree. Therefore, HU causes drastic changes in lymphocyte numbers.

Table 5. Effect of Hindlimb Unloading on Splenocytes and Thymocytes*

	Spleen (Days Unloaded)				Thymus (Days Unloaded)			
	Control	2d	4d	10d	Control	2d	7d	10
No of Mice	8	5	5	10	8	5	5	6
CD4 ⁺ CD8 ⁺	—	—	—	—	93.9 ± 12.6	29.5 ± 18.4	24.8 ± 7.5	22.0 ±
CD4 ⁺ CD8 ⁻	25.4 ± 4.4	15.1 ± 3.1	11.5 ± 3.6	10.1 ± 1.3	14.8 ± 2.9	12.6 ± 3.6#	5.8 ± 1.4	3.2 ±
CD4 ⁻ CD8 ⁺	14.8 ± 2.7	7.8 ± 1.8	7.6 ± 2.3	7.6 ± 1.4	5.4 ± 1.8	4.1 ± 1.0#	1.9 ± 0.8	0.9 ±
CD19 ⁺	52.7 ± 6.7	41.9 ± 4.9	27.3 ± 7.0	19.9 ± 5.3	—	—	—	—

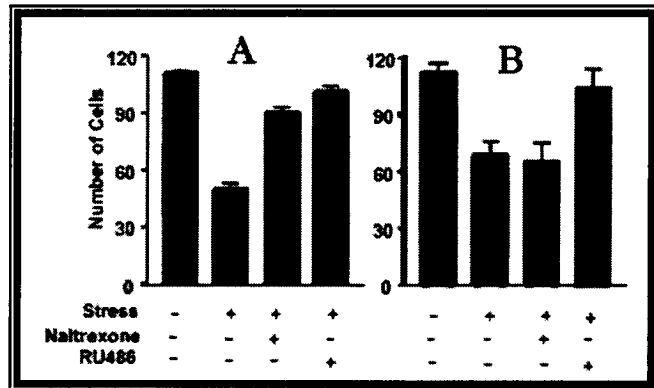
*Male Balb/c mice at 8 weeks old were subjected to hindlimb unloading for different times and cell subpopulations were analyzed by flow cytometry. The values represent total number of cells of each phenotype (mean ± SD, x 10⁶). Student's t test revealed significant reductions at time points as compared to control. p<0.01, except marked by #.

The Role of Endogenous Opioids. Our previous investigations revealed that restraint stress-induced splenocyte reduction requires endogenous opioids, since blocking opioid receptor with naltrexone or naloxone abolished this effect. This result was recently corroborated using μ-opioid receptor knockout mice. To determine whether endogenous opioids play a similar role in

the lymphocyte reduction seen in HU, we treated Balb/c mice with naltrexone (20 mg/kg daily) during HU for 2 days. We found that naltrexone dramatically inhibited splenocyte reduction (Fig. 21A). Treatment with RU486, a steroid receptor antagonist, similarly prevented splenocyte depletion, an interesting observation considering that some effects of endogenous opioids have been shown to be exerted through the induction of corticosteroid production. It is also possible that endogenous opioids and corticosteroids each act separately on splenocytes. In any case, the link between corticosteroids and endogenous opioids in the modulation of splenocyte homeostasis warrants further investigation. In contrast to the spleen, thymocyte reduction after HU was blocked only by RU486, not by naltrexone (Fig. 21B), indicating a critical role for

corticosteroids in this process. This is in agreement with the reported key role of corticosteroids in opioid-mediated thymocyte reduction. Thus, HU-induced reductions in splenocytes and thymocytes are each regulated by different mechanisms. Similarly, we have shown that blocking the Fas-FasL interaction interferes with the depletion of splenocyte, but not thymocytes (see below).

Figure 21. The role of endogenous opioids in hindlimb unloading-induced lymphocyte reduction. Eight-week-old male Balb/c mice were subjected to hindlimb unloading for 2 days. Naltrexone (10 mg/kg) or RU486 (20 mg/kg) were administered intraperitoneally 3 hours prior to hindlimb unloading. Total leukocytes (x 10⁶) in the spleen (A) and thymus (B) were enumerated (n=4).



The Effect of Blocking the Fas-FasL Interaction in Hindlimb Unloading-Induced Lymphocyte Reduction. We previously showed that blocking the interaction of Fas and FasL inhibits restraint stress-induced lymphocyte reduction, a result confirmed by a recent study. Similar to what we observed in restraint stressed mice, we found that HU also induces Fas expression as detected by northern blot analysis and flow cytometry (unpublished data). To investigate the effect of increased Fas expression, we used Fas-Fc fusion protein or antibody to interfere with FasL, and found that both agents prevented HU-induced splenocyte reduction (Fig. 22 and data not shown). Similar results were obtained with anti-FasL (MFL3) in 2 experiments.

Interestingly, blocking FasL activity had no effect on thymocyte reduction in these mice. It should be pointed out that after a full week of hindlimb suspension, this intervention is dramatically less effective (data not shown), indicating that besides the Fas and FasL interaction, there must be additional mechanism(s), such as free radicals, that also control lymphocyte viability.

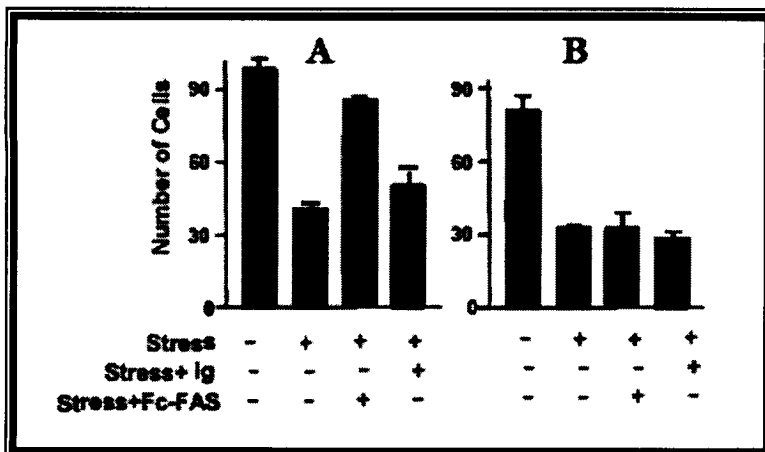


Figure 22. Blocking the Fas-FasL interaction prevents HU-induced lymphocyte reduction. Eight week-old male Balb/c mice were hindlimb unloaded with or without administration of Fas-Fc or control Ig (150 ug per mouse). Total cells (x 10⁶) in the spleen (A) and thymus (B) were counted (n=4).

Free Radicals Sensitize Splenocytes to Fas-mediated Apoptosis. It has been reported that free radicals are important in stress-induced reductions of lymphocyte. We have observed that splenocytes from restraint stressed mice have increased sensitivity to JO2 (Fas activating) antibody-mediated apoptosis *in vitro*, an effect attributed to increased Fas expression by splenocytes. Also, we have recently shown that splenocytes and thymocytes from hindlimb-unloaded mice are more sensitive to dexamethasone-mediated apoptosis *in vitro* and that their increased susceptibility to cell death could be blocked by the anti-oxidant, N-acetyl-cysteine (data not shown). In light of our discovery of the role of Fas and FasL in stress-induced lymphocyte apoptosis, we hypothesized that free radicals produced during stress conditions promote Fas-induced apoptosis. To test this hypothesis, we exposed splenocytes to H₂O₂ and

JO2 antibody, either alone or together, and measured subsequent apoptosis by DNA content analysis by flow cytometry. We found that when either H₂O₂ or JO2 were applied alone, the effect was minimal. However, when splenocyte cultures were treated

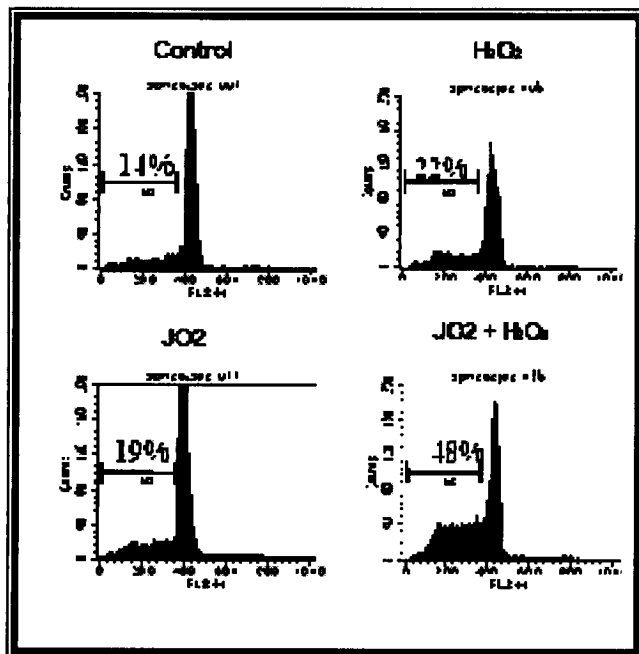


Figure 23. The synergistic effect of Fas agonist and H₂O₂ in the induction of apoptosis in lymphocytes. Freshly-isolated splenocytes were treated with H₂O₂ (25 nM), JO2 antibody (100 ng/ml), or both for 16 hours. Apoptosis was determined by DNA content analysis. The subdiploid region represents apoptotic cells.

concurrently with both reagents, there was a significant increase in the number of cells undergoing apoptosis (**Fig. 23**), indicating that signaling via Fas and the action of

oxidative molecules synergistically amplify apoptosis. This finding demonstrates an important link between Fas-mediated apoptosis and sensitivity to free radical in stress-induced lymphocyte losses. Further investigation of the molecular mechanisms involved will lead to a better understanding of how stress affects the immune system.

The Effects of Hindlimb Unloading on Lymphocyte Mitogenic Response The ability of activated lymphocytes to proliferate is essential in promoting an effective immune response. This capacity may be diminished under conditions of space flight. We used our HU model to determine if there is an effect on the proliferative response of lymphocytes. We subjected mice to various durations of HU, then examined the proliferation of splenocytes in response to anti-CD3 antibody *in vitro*. Equal numbers of cells from mice subjected to unloading for different times were added to 96-well plates and stimulated with anti-CD3 and their proliferation assessed by 3H-thymidine incorporation. As shown in **Figure 24A**, HU significantly reduced the proliferative response of splenic T cells. It is interesting to note that the most significant effect occurred after only 2 days of unloading treatment: a 70% decrease compared to control, even though equal cell numbers were plated. To determine the role of the Fas-FasL interaction in the suppression of cell growth, Fas-Fc was added to some cultures. As shown in **Figure 24B**, Fas-Fc

almost completely restored normal proliferation, demonstrating that FasL is essential in the HU induced suppression of T cell mitogenic responses.

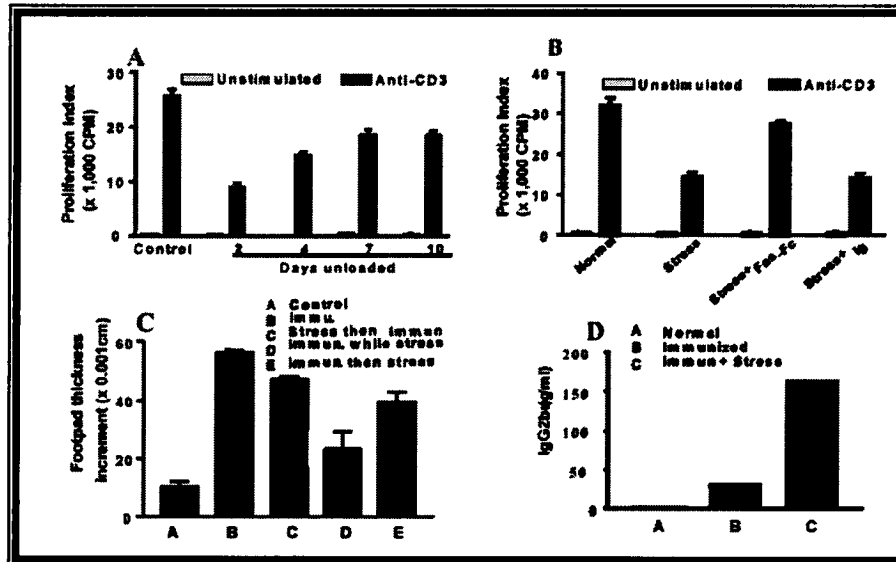


Figure 24. The effect of hindlimb unloading on immune responses. (A) Balb/c mice were subjected to hindlimb unloading of varying duration. Equal numbers of splenocytes were cultured in 96-well plates and stimulated with anti-CD3. Proliferation was measured by ^3H -thymidine incorporation. (B) Eight week-old Balb/c were subjected to hindlimb unloading with/without administration of Fas-Ig (150 ug per mouse) or control Ig. Anti-CD3 induced splenocyte proliferation was determined. (C) Mice were primed with OVA in CFA and then challenged with OVA in IFA at different times relative to hindlimb unloading. DTH was induced by injecting particulate OVA into the right footpad, while the left footpad received PBS as a control. The magnitude of swelling was measured. (D) Mice were treated as in (C) and serum concentration of OVA specific IgG2b was determined by ELISA.

We also examined the effect of HU on antigen-specific immune responses *in vivo*. Mice were first primed with ovalbumin (OVA) in complete Freund's adjuvant (CFA), then challenged with particulate OVA in incomplete adjuvant (IFA) either during HU, or 2 days before starting or 2 days after ending the treatment. The DTH response was measured by changes in footpad thickness 24 hours after challenge. We found that the effect of HU on OVA-induced DTH response varied with the temporal relationship between injection and treatment (Fig. 24C). The most significant depression of DTH was observed when antigenic challenge and unloading were administered at the same time. This effect correlated with attenuation of increases in spleen size, and concurrent immune challenge and unloading treatment nearly eliminated mitogenic splenomegaly. Thus, during HU, the capability of the cellular immune system to respond to antigenic challenge is significantly diminished. This is in agreement with the recent study reporting that hindlimb-suspended mice are high susceptible to infection with *Klebsiella pneumoniae*, in which the Th1 response is critical in resistance.

We have started to determine Th2 type humoral immune response by measuring antibody production. Our preliminary data show that OVA specific IgG2b production is increased in unloaded mice (Fig. 24D). We are in the process of determining the production of other class of immunoglobulins. Though these data are preliminary, they suggest that stress inhibits Th1 type immune response and increase Th2 type immunity.

Recovery of Lymphocytes in Spleen and Thymus after Hindlimb Unloading. It is well established that various types of stress lead to lymphocyte reduction. Little is known about the recovery process, however. To begin to address this important area, we examined the recovery of lymphocyte numbers following treatment of mice with HU. Mice were subjected to HU for 4 days and allowed to recover under normal housing conditions. After various periods of recovery, mice were euthanized and splenocytes and thymocytes were enumerated (**Fig. 25**). We found that the overall recovery is quite rapid, with splenocytes recovering sooner than thymocytes. Significant splenocyte recovery was observed on day 2, and full recovery by day 7, while full

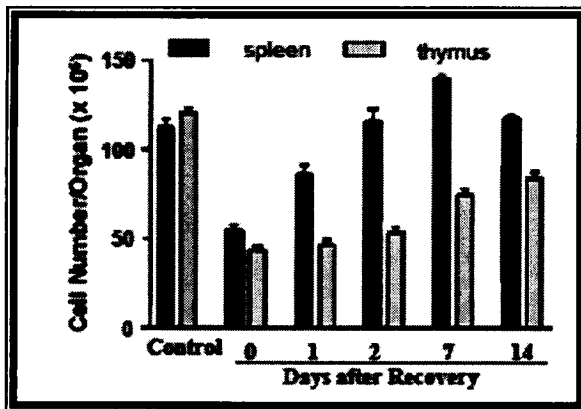


Figure 25. Recovery of splenocytes and thymocytes after hindlimb unloading. Eight-week-old male Balb/c mice were subjected to hindlimb unloading for 4 days and the cellularity of the spleen and thymus determined after various periods of recovery. (n=4)

recovery of thymocytes took much longer. This is a rather surprising result, as we expected thymocytes to recover first, then repopulate the peripheral lymphoid organs. It is possible however, that the delayed recovery of thymocytes is due to increased export. Conversely, the faster recovery of splenocytes could be due to immigration of cells from other peripheral sites. Nevertheless, this is an important issue and a detailed understanding would contribute to ways of improving the health of astronauts after returning from space. Therefore, we have proposed experiments to investigate this area.

Radiation Synergizes with Fas-signaling and Hindlimb Unloading to induce Apoptosis

Astronauts in space are exposed to various stresses in addition to radiation. While much is known about the effects of stress and radiation, individually, on the immune system, their combined effects have not been thoroughly investigated. Since we have shown that HU-induced lymphocyte apoptosis is probably due to upregulation of Fas expression, we looked first at the effect of radiation on the sensitivity of T cell hybridoma A1.1 cells to apoptosis induced by Fas agonist antibody JO2. As shown in **Figure 26A**, while irradiation with 2 Gy gamma rays induced no apoptosis in these cells (A1.1 cells can tolerate up to 8 Gy), irradiated cells were much more sensitive to JO2-induced apoptosis. To investigate whether radiation synergizes with HU stress, we subjected mice to HU for 2 days and then exposed them to 2 Gy gamma radiation and continued HU for 6 more hours. The presence of apoptotic cells in the spleen and thymus was determined by subdiploid DNA content. As shown in **Figure 26B**, splenocytes and thymocytes from suspension-treated mice were more sensitive to radiation showing a higher percentage of apoptosis after combined treatment compared to either treatment alone. It is important to emphasize that mice have a strong phagocytic capacity to remove cells at the early stages of apoptosis. Consequently, apoptotic cells seen in our analyses are the ones that have overwhelmed the phagocytic capacity. These results demonstrate that radiation synergizes with hind limb unloading stress to induce high levels of apoptosis in lymphocytes. We will further investigate the role of Fas in this process *in vivo* and verify whether high energy particle radiation also synergizes with stress.

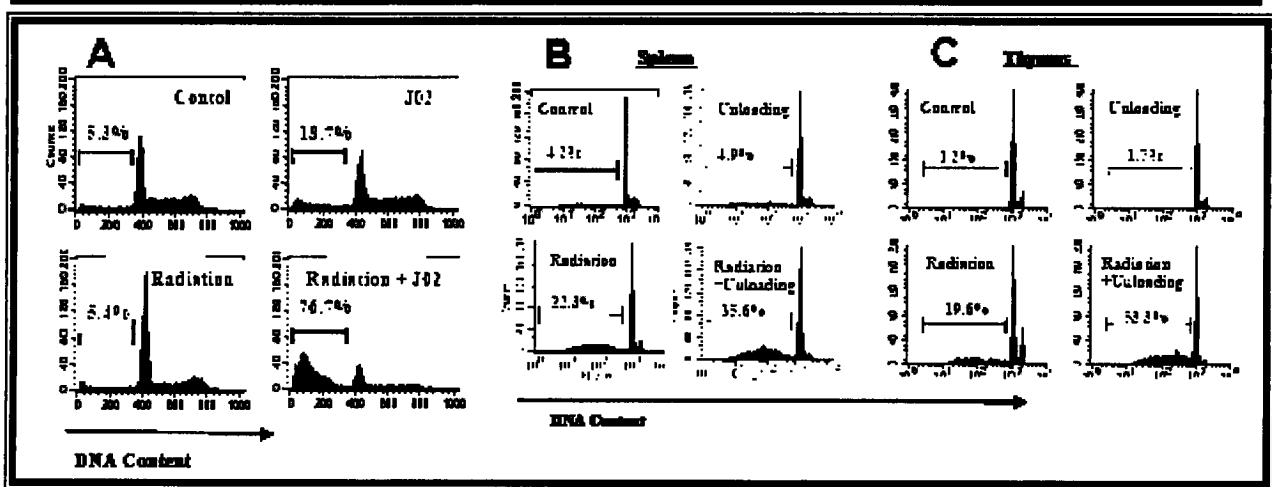


Figure 26. Radiation Synergizes with Fas-signaling and Hindlimb Unloading to induce Apoptosis. (A) Radiation promotes Fas-induced apoptosis. T cell hybridoma A1.1 cells were gamma-irradiated (2 Gy), then cultured for 16 hrs in the presence or absence of anti-Fas antibody JO2 (100 ng/ml). Apoptosis was measured by DNA content analysis. **(B,C)** Unloading stress enhances lymphocyte apoptosis induced by irradiation. Eight-week-old Balb/c mice were subjected to hind limb unloading for 48 hrs and then gamma irradiated (2Gy). After another 6 hours of unloading treatment, apoptosis in freshly-isolated splenocytes **(B)** and thymocytes **(C)** was measured by DNA content analysis.

Publications:

- **Shi YF**, Devadas S, Greeneltch KM, Yin DL, Mufson RA, Zhou JN. Stressed to death: implication of lymphocyte apoptosis for psychoneuroimmunology. *Brain Behav Immun* 17:S18-S26, 2002.
- Zhang XR, Zhang L, Li L, Glimcher LM, Keegan AD, **Shi YF**. Reciprocal expression of TRAIL and CD95L in Th1 and Th2 cells: role of apoptosis in T helper subset differentiation. *Cell Death Differ*, in press, 2003.
- Greeneltch KM, Haudenschild CC, Keegan AD, **Shi Y**. The opioid antagonist naltrexone blocks acute endotoxic shock by inhibiting tumor necrosis factor- α production. *Brain Behav Immun*, submitted in revised form per editor's request, 2003.

Presentations: Abstracts/Lectures

- **Shi YF**. The role of endogenous opioids and corticosteroids in the reduction of splenocytes and thymocytes induced by hind limb suspension. *Bioastronautics Investigators' Workshop*, Galveston, TX, January 13–15, 2003.

Project 6: George E. Fox, Ph.D., Principal Investigator; "Microorganisms in the Spacecraft Environment" (addresses Strategic Plan Risks 2-5 and Critical Questions 7.06, 7.09, 7.11, 7.13)

1. **Microbial ecology of the spacecraft environment.** Our goal was to develop the basis for characterizing and monitoring the important microbial components of spacecraft air, water and surface samples with emphasis on early detection of increased levels of undesirable

organisms or indicator organisms. Due to budgetary reductions and progress in other areas we focused this area on water, as it will ultimately be straightforward to extend the technologies to other samples. Substantial progress was made towards this objective.

We successfully developed the foundations for a water-monitoring system based on 30 oligonucleotide probes, **Table 6**, that target the 16S ribosomal RNA (rRNA) molecule and identify bacterial contaminants as either members of general groups or at the genus and species level.

Table 6: Validated Probes for Use in Molecular Beacon Assays

Probe	Target organism(s)	Sequence (5'-3')	<i>E. coli</i> 16S rRNA Positions	Predicted T _m (°C)
Acineto414	<i>Acinetobacter</i>	GCTTTACAACC(A/C)(A/T)AAGGCCT	414-33	59.4
A6	<i>Acinetobacter</i>	TAGTGAACACTGGAA	165-79	42.7
Acineto437	<i>Acinetobacter</i>	AGCCTCCTCCTCGTTAAA	437-55	58.9
Burk803	<i>B. cepacia</i>	CAT(C/G)GTTTAGGGCGTGGAC	803-21	64.8
Burkchap	<i>B. cepacia</i>	TTCCGGTACCGTCATCCC	444-61	61.5
A7	<i>B. cepacia</i>	GACTCTCCGCCCTCACG	1006-22	64.9
Bc_probe	<i>B. cepacia</i>	GACTCTCCGCCCTCTCACG	1006-24	63.1
Burk probe ^c	<i>B. cepacia</i>	CCTCTGTTCCGACCA	1240-54	50.2
A3	<i>V. proteolyticus</i>	TAGCCGCAGCTCG	99-111	56.1
A4	<i>V. proteolyticus</i>	TCAATTATCTACGTAAT	100-16	49.2
A5	<i>V. proteolyticus</i>	TGCGAGAGTGAAACACC	1464-80	58.3
V3VPR ^d	<i>V. proteolyticus</i>	CGCTAACGTCAAATAATGCATCTA	467-90	57.6
Pseud439	<i>Pseudo. sp</i>	TGCCCTTCCTCCCAACTT	440-57	69.5
Pa642	<i>P. aeruginosa</i>	ATCTCTAGCTCAGTATT	642-54	36.0
E1	<i>E. coli</i>	AGCAAAGGTATTAACCTTTACTCCCCT	452-76	61.6
E2	<i>E. coli</i>	TTCCCGAAGGCACATTCT	1019-36	58.9
Eco71	<i>E. coli</i>	GCAAAGCAGCAAGCTC	71-86	58.3
E3	Gram negatives	TTCCCGAAGGCACCAATC	1019-36	61.8
P1	Gram positives	ATGTTCTCCCTAATAACAGAGT	421-43	55.3
P2	Gram positives	GGAAGGCUCUAUCUCUAGAGUUGUCAA	1000-27	63.7
P3	Gram positives	GGGTGTTACAAACTCTCG	1399-16	53.7
Ent2	Enterics	TAGTTATCCCCCTCCATCAGGCAGT	131-57	67.0
Ent3	Enterics	AGCTACCGTTTCCAG	156-70	45.5
Ent5	Enterics	ATCCGATGGCAAGAGGC	213-29	59.4
16S-R	All bacteria	AGAAGGAGGTGATCCACGCA	1522-41	59.4
Pan339	All bacteria	CTGCCTCCCGTAGGAG	339-54	54.5
Pan780	All bacteria	AGGGTATCTAATCCTGTTT	780-98	47.1
16S ^e	All bacteria	AAACTCCTACGGGAGGCAGTAAGGAAT	336-65	68.1
Eco898 ^f	All bacteria	ACTCCTACGGGAGGCAGC	898-916	60.2
Eub338	All bacteria	GCTGCCTCCCGTAGGAGT	338-55	60.5

^aT_m calculated using online calculator at <http://alces.med.umn.edu/bin/newrawtm>

The entire set of probes was validated such that they all work at the same conditions (temperature (42°C), concentration, wash protocols, etc.). This is an important feature that ensures universal operation conditions and facilitates sample handling as well as application instrument design. Candidate probes were originally suggested by sequence comparisons and/or published studies and the utility of each was individually evaluated. Many candidate probes were rejected. Rejection occurred for a variety of reasons including dependence on high or low

hybridization temperatures or extremely stringent wash conditions for specificity. The probe set has been tested using RNA from pure cultures as well as environmental samples, including Martian and Lunar soil simulants. The limit of detection for most probes was around 20 ng of target RNA using standard Northern blot protocols, and 0.1 µg of total RNA using solution assays. We have begun to apply these probes in molecular beacons and microarrays as described further below.

During the course of our investigations, NASA began to return microbiological samples from the International Space Station. Approximately two hundred partial 16S rRNA sequences have been determined by Dr. Duane Pierson's group at NASA-JSC from bacteria that grew on culture media. Future studies will include unculturable organisms, too. This is important data for the work being conducted here because it provides an understanding of what normal populations are, as well as precise insight into which genera to monitor in order to detect changes in population structure. We therefore obtained this sequence information from Dr. Pierson in project year 3 and constructed alignments and phylogenetic trees. A very limited number of organisms were identified and, as expected, many were organisms frequently associated with human populations including some potentially pathogenic genera. The dominant genera, with number of isolates indicated in parentheses, were *Acinetobacter* (14), *Bradyrhizobium* (15), *Burkholderia* (7), *Methylbacterium* (14), *Pseudomonas* (16), *Ralstonia* (22), *Sphingomonas* (31), *Staphylococcus* (51), *Bacillus* (32), and *Micrococcus* (14). In most cases, 2-4 species were observed within each common genus. For example, there were 21 isolates of *Staphylococcus epidermis* and 12 isolates of *S. aureus*. In contrast, lone isolates were rare. Given the large number of repetitive isolates this strongly suggests that the survey is a good representation of the entire ecosystem from the perspective of cultivable organisms and hence a good starting point for design of our future arrays in anticipation of a more comprehensive survey that includes the potentially large numbers of non-cultivable bacteria.

2. Detection of pathogens arising from unexpected directions. Our goal was to develop the theoretical basis for the design of a hybridization array that could characterize the phylogenetic position of any bacterium present in a sample, regardless of prior expectations of what might be found, a "universal classifier". If this were successful, we would then seek to develop the framework for implementing the signature information into a hybridization array assay. This objective was accomplished in its entirety, and attracted considerable press attention (e.g., *The Scientist*, *ASM News*, *The Lancet Infectious Diseases*).

Microbial monitoring based on traditional 16S rRNA targeted probe design suffers from two difficulties. The first and most severe is that one does not necessarily know what the problem organism is going to be, because the microbial population of the environment is complex, and little is known about the effects of long-term exposure to the space environment on bacterial pathogenesis. Therefore, a persistent microbial problem might arise from a usually non-problematic organism that may or may not have been previously cultured. Secondly, strains of the target organism may be present that happen to not have the probe target sequence.

During the course of the funding period we recognized that if one sought to characterize the phylogenetic position of a target organism rather than its precise identity, a far more general

monitoring system was possible. Since bacterial phylogeny is readily recovered from 16S rRNA sequence comparisons, it is clear that aligned 16S rRNA sequences must contain many highly informative individual sites. We hypothesized one could find extended oligonucleotide sequences that would be highly characteristic signatures of particular phylogenetic groupings. The phylogenetic grouping encompassed would be different for each signature sequence and some would be somewhat noisy, in that the characteristic nucleotide is absent in some organisms that belong to the cluster of interest and present in some organisms outside the cluster. Thus, beginning in year 1 and extending into year 2, we developed a quality index to measure the extent to which any particular short sub-sequence is a signature of a particular node in a representative phylogenetic tree. We also developed an algorithm to specifically identify all useful signature sequences of selected length N. In so doing we proved the hypothesis by finding that in fact thousands of signature sequences of length 11 or larger existed and that these sequences address more than 70% of the nodes in a representative phylogenetic tree. We established a database of these signature oligonucleotides, which is located at http://prion.bchs.uh.edu/16S_signatures and in a more useful format at <http://prion.bchs.uh.edu/Signature16S/index.html>. In designing probes one could thus find target signature sequences that are highly characteristic of all strains of a species, e.g. *E. coli* specific probes or all species of *Staphylococcus* - a genus-specific probe. Of greatest interest, however, is that one could make an array of numerous probes targeting signature sequences representing all major and minor bacterial divisions with multiple probes addressing each target grouping. Depending on the number and types of probes included, arrays of varying focus and classifying ability can be envisioned. We are implementing signature arrays (see Section 4 below). (Zhang, et al., 2002; see p. 49.)

The original approach used a signature quality index, which ranged from 0-1 to calculate the value of any 16S rRNA subsequence of length N as a signature of a particular cluster in a phylogenetic tree. When signature quality is defined in this way each candidate subsequence gives a positive score for every grouping that contains it at least once. In actual hybridization experiments, longer (15 plus nucleotides) probes are preferable. However, as N increases, exact match signature sequences increasingly target tip clusters rather than the interior clusters of the tree. From a practical perspective it is not actually necessary that the signature sequence always be exactly present in the organisms that belong to the target cluster. Instead, it is sufficient that the candidate signature subsequence be similar enough in all/most members of a target group that it can be detected by hybridization despite the minor variations. All subsequences of length N that meet this criterion will be useful if they meet a second criterion which requires more differences in non-members of the target cluster. Thus, we recently completed the development of an "in/out" algorithm that allows us to design signature probes of length 45 for approximately 45% of the interior nodes in the representative tree.

The presence of large numbers of signature sequences raised the obvious question of whether or not they could be directly generated and measured by experiments. Fragments can be readily produced in two ways, restriction digestion of rDNA or enzymatic digestion of 16S rRNA by RNase T₁ or RNaseA. The latter likely would be experimentally easier, and we are implementing this strategy with custom DNA probe arrays. Given Dr. Richard Potember's (current member of the NSBRI Technology Development team) efforts to develop a miniaturized mass spectrometer for use during space flight an obviously interesting choice for detection would be mass determination. We therefore examined the problem *in silico* taking into account two

experimental difficulties, the limited alphabet (no interior G) of RNase T₁ and the near mass identity of C and U. It was found that RNase T₁ digestion of 1,921 completely sequenced 16S rRNAs gives a set of 8,938 distinct oligonucleotides of size ranging from 2 to 54 nucleotides. These oligonucleotides have 1,077 distinguishable masses. On average, the 16S rRNA of an organism from the 1,921 prokaryotes under consideration gave 130 distinct oligonucleotides, which have 79 different molecular weights. Approximately one-sixth of the organisms could be identified in a hypothetical very complex mixture by just a single mass peak, and most organisms can be unequivocally identified by a combination of the presence of a few fragments. Thus, if the composition of the in flight microbial flora is narrow as the initial results suggest (see previous section) a mass profile of a sample containing 3-5 dominant bacterial species will contain approximately 40 high mass peaks (from oligonucleotide products of length 10 and larger). These will typically be resolvable such that individual organisms can be identified. In addition, most oligomers in the 10-16 base size range have considerable signature value. Therefore, if an organism is present whose 16S rRNA sequence is not known it will likely still be possible to deduce its general phylogenetic position from the data. Moreover, the profile obtained may be a good immediate picture of the normalcy or lack thereof of the entire microbial population. In view of these very promising results, we began efforts to use mass spectrometry as a monitoring tool, using the excellent MALDI-TOF instrument available to us on the UH campus.

3. Differences in gene expression by microorganisms under modeled microgravity. The low gravity/high radiation environment associated with manned space flight may modify cellular behavior in ways that, over extended time, select for changes in bacterial populations and/or behavior. There is concern that such changes may lead to increased or altered pathogenicity of bacteria. The goal of these experiments was to begin the Earth-based studies needed to assess the significance of such risks. The immediate goal was to directly test the effects of low shear modeled microgravity (LSMMG), as obtained by the use of the rotating wall vessel bioreactor (RWV) originally designed at the NASA Johnson Space Center, on microbial physiology at the level of gene expression. The expression of each gene is individually monitored by Sigma-Genosys Panorama cDNA arrays. It was expected that there would be specific genes that are responsive to environmental perturbations associated with LSMMG. The goal was to identify them and formulate hypotheses as to why the LSMMG environment was invoking such changes. The reviewers were less enthusiastic about this area of the work than others and we therefore de-emphasized the amount of effort devoted to it, but nevertheless made substantial progress.

Initially, we conducted hybridizations on shake flask cultures of *E. coli* grown on either minimal media or yeast extract to obtain a baseline understanding of the amount of expression variation seen between replicate experiments and replicate hybridizations in order to understand how large a difference must be to be statistically significant. This work was done in collaboration with Dr. Yuriy Fofanov, a biostatistician in the University of Houston Computer Science Department. Subsequently, we have resolved minor experimental design difficulties (e.g. elimination of gas bubbles) and begun experiments in the RWV with *E. coli*. Experimental controls include a 1xg RWV experiment in which the reactor is rotated so as to randomize the gravitational vector, and use of soluble, alternate terminal electron acceptors (e.g., nitrate), to avoid mass-transfer limitations. Functional genomic analysis, comparing LSMMG and the 1 x g RWG control, identified 18 genes that were far more highly expressed in LSMMG, and 26 genes far more highly expressed in the 1xg RWG control. Among the defined genes with increased transcription in response to LSMMG, are genes involved in the *E. coli* acid tolerance response

system (transcriptional gene regulators *yhiE* and *yhiF*, the putative chaperone *hdeA*, and the associated genes *hdeB* and *hdeD*), genes involved in cell motility (*flg* and *fli* genes), and chemotaxis regulating genes (*cheZ* and *tar*). Induction of acid tolerance response genes in minimal media (pH 7.4) under LSMMG could indicate that they are involved in a general *E. coli* stress response pathway. Increased transcription of the flagellar, acid response, and chemotaxis genes in LSMMG, suggests zones of low nutrients and high metabolite concentration are occurring in the LSMMG similar to those theorized to occur in space. Further studies are ongoing with *E. coli* cultures growing under anaerobic conditions and initial standardization experiments have begun with *Bacillus subtilis*.

4. Spacecraft-compatible methodologies for use in microbial detection. The goal was to identify chemistries and methodologies for nucleic acid isolation and detection with emphasis on suitability for implementation in space-borne monitoring systems. In particular, we planned to develop novel methodologies in support of Aims 1-3 above. Substantial progress was made as summarized below and the scope of this effort was expanded to include mass spectrometry.

a. *Immobilized metal affinity chromatography (IMAC):* We demonstrated that IMAC matrices, which are usually used with proteins, could also adsorb single-stranded nucleic acids through metal ion interactions with aromatic base nitrogens. Oligonucleotide duplexes, plasmid and native genomic DNA show low IMAC binding affinity, while RNA and single-stranded oligonucleotides bind strongly to matrices such as Cu(II) iminodiacetic acid (IDA) agarose. The affinity of yeast RNA for IDA-chelated metal ions was found to decrease in the order: Cu(II), Ni(II), Zn(II), and Co(II). Adsorption isotherms for 20-mer oligonucleotide homopolymers showed that purines are strongly favored over pyrimidines and that double-stranded duplexes are not bound.

We demonstrated metal chelate affinity separations of nucleic acids were very successful in removing primers and imperfect products from PCR reactions, and would probably also be applicable to cleanup of NASBA reaction mixtures used to amplify microbial and/or viral RNA for easier detection. Analytical (hybridization assay) possibilities are raised by separation of 20-mer oligonucleotide duplexes containing centered single-base mismatches. Finally, the low affinity of structured rRNA for these matrices suggests the separation of rRNA from fragments, mRNA, etc. Active development and spinoff licensing discussions are underway with two different companies, and an application for development funding (leveraging the NSBRI investment), has been invited by one of the largest nucleic acid technology companies. Two recent developments, which are still being pursued, are the enhancement of binding by lowering of water activity with osmolytes (affinity has been tripled in preliminary experiments), and use of selective renaturation to create “affinity handles” in structured nucleic acids (genomic and rRNA) for selective purification.

b. *Molecular beacons and fluorescent nucleotide analogs:* Self-quenching hairpin probes are especially promising for use in space flight because they allow separation-free homogeneous assays of high specificity. We have developed a set of self-quenching molecular beacons specific to bacterial ribosomal RNA, and demonstrated sensitive, specific detection of target organisms even against a high background of unrelated rRNAs. We have shown that probes developed in a non-beacon format can be transformed into beacons with the use of appropriate design tools to avoid self-hybridization and to optimize stem stability. This is important, as we possess an

extensive set of validated conventional probes against organisms of proven space flight relevance.

We also developed a very simple assay combining simple heat lysis and beacon reporting, and demonstrated the compatibility of beacon reporters with low-toxicity detergent lysis. These seemingly-prosaic practical advances provide an increasingly powerful “tool box” for developing convenient, low-energy-use, low-toxicity assays applicable to routine monitoring of the microbial population of the spacecraft environment. More recently, we have shown that hybridization-responsive DNA probes can also be based on highly fluorescent base analogs, which are quenched or dequenched upon transfer from a single- to a double-stranded environment. Probes containing fluorescent base analogs such as 2-aminopurine (2AP) can be prepared much more conveniently than traditional beacons, and can be designed such that fluorescence intensity either increases or decreases upon hybridization. The thermal stability of 2AP-containing duplexes is significantly enhanced, allowing increased selectivity compared to unmodified DNA probes. Also, 2AP is readily incorporated into DNA oligonucleotides during solid-phase synthesis, avoiding the expensive coupling and purification steps needed for attachment of exogenous fluorophores. Sequence-selective assays can be developed in which 2-level reporting (fluorescence restoration or quenching) would be more informative than detection based solely on fluorescence enhancement.

c. Mass spectrometry identification: Given the promising theoretical observation that highly characteristic signature sequences of ten or more nucleotides can be generated by a simple nuclease digestion, and then recognized by their masses, we sought to learn more. Koomen *et al.* (2002) have published an extensive review of the resolution requirements for accurately determining oligonucleotide composition. They determined that all compositions of oligomers up to 13mers could be accurately assigned at 5ppm mass accuracy or less. Such accuracy is achievable in current MALDI-TOF spectrometers.

In order to implement the technology into our laboratories, we began by analyzing a short synthetic 19-base oligonucleotide of sequence 5'-CCCCUUGAUAGCCGCUACG_{OH}-3' that was treated with one unit of T₁ ribonuclease for 15 min. at 37 degrees C. Two other oligomers were also added to the mixture following digestion to act as external calibration references. **Table 7** shows the expected and measured m/z values after calibration by forcing the 19mer starting material and two external references to their known values. This experiment was done under undercutting conditions in order to maximize the complexity of the spectrum so that we could assess a variety of product types. Thus, some of the original 19-mer oligonucleotide remained undigested serving as a second standard and fragments that still contained internal guanosines and cyclic phosphates were obtained. In actual analyses, complete digestions will be used which simplifies the spectrum considerably. The mass range of interest in 16S rRNA samples will be from approximately 3200-6000. It is clear from the results obtained to date that fragments of this size can be measured with considerable accuracy.

MALDI-TOF spectra can be complicated by the mixture of cation adducts, primarily Na⁺, K⁺, and NH₄⁺, which bind with high affinity along the phosphate backbone of the RNA and produce a mixture of daughter peaks of the parent mass. This problem has been alleviated by various techniques. We have found that the most effective and easily implemented methods include the following: (1) exchange of ammonium salts for sodium ions prior to isopropanol precipitation

following initial RNA isolation; (2) resuspension in RNase free deionized water with no buffer salts added; (3) reverse phase binding to a C18 ZipTip™ (Millipore) in the presence of tetraethyl ammonium acetate, followed by desalting and elution directly with a MALDI matrix formulation with a diammonium citrate additive; and (4) acquiring spectra in negative ion mode.

Table 7:

19mer starting material

5'-CCCCUUG/AUAG/CCG/CUACG-3'

Sequence (5'-3')	expected m/z [M- H-]	meas. after calibration	Sequence (5'-3')	expected m/z [M- H]	meas. after calibration
CCCCUUG/AUAG/CCG/CUACG _{-OH}	5971.63	5971.48	CCCUUG>p	2177.27	2177.21
CCG>p	954.57	954.97	CCG/CUACG _{-OH}	2483.47	2483.66
CCCC _{-OH} *	1157.59	1157.59*	CCCCUUG/AUAG>p	3487.07	3487.39
AUAG>p	1308.79	1309.02	AUAG/CCG/CUACG _{OH}	3793.3	3793.3
CUACG _{-OH}	1527.99	1527.77	14mer*	4421.73	4421.73*

*indicates external reference oligo

>p indicates 2'-3' cyclic phosphate

-OH indicates the 3'-OH of the 19mer starting material

We have also begun development of a macromolecule-inaccessible deionizing matrix which may be very useful in a wide variety of sample-preparation applications. The inclusion of ammonium salts at every possible step has been shown to displace other cation adducts. During the MALDI ionization process, the protonated, or lowest [M-H]⁻ peak then dominates, thereby reducing the complexity of the spectrum. With these technological improvements in place we have recently begun studies with single 16S rRNAs and will subsequently examine the complexities introduced by mixtures.

d. Signature arrays for identification of unexpected problem organisms: Our demonstration of the existence of vast numbers of signature sequences within the 16S rRNA dataset led us to extend our original goals in this area and develop such a signature array. Such an array would score the presence/absence of large numbers of signature sequences in a bacterial sample and thereby allow one to deduce the phylogenetic affinity of the major component organisms in a sample. In order to obtain a functional final chip design of this type we need to test tens of thousands of potential probes that target signature sequences to various key nodes in the phylogeny. In order to do this, we are developing methods of array hybridization for 16S rRNA fragments with custom DNA probes arrays prepared for us by Xeotron, Inc., a University of Houston spinoff company. The key advantage of Xeotron chips is that oligomers are synthesized on the chip without requirement of a photomask. The company can provide custom chips containing up to 8000 probes in days at a cost of approximately \$360/each. The arrays can be reused at least once and we are currently seeking methods to increase the number of repeat uses in order to decrease array cost further.

Heating in the presence of a magnesium buffer is used to randomly fragment 16S rRNA, thereby facilitating hybridization. The fragmented RNA is labeled using the Ulysis Nucleic Acid Labeling Kit (Molecular Probes, Eugene, OR), which uses a proprietary platinum dye complex (Alexa Fluor 647) to form a stable adduct with the N7 position of guanine (and to a lesser extent,

adenine) bases. We have developed protocols that allow use of our Agilent 2100 Bioanalyzer lab-on-a-chip CE instrument for high-resolution analysis of the labeled rRNA fragments despite its dependence on background fluors for detector focusing.

The fragmented, labeled 16S rRNA samples are hybridized to our custom signature probe chips using Xeotron's specialized Microfluidic Station hybridization apparatus, which provides temperature control and mixing shear to promote efficient mass transfer and hybridization by reciprocating flow through the MEMS microchannels of the chip. The labeled sample is circulated and binds to the probe DNA on the Xeotron chip during 2 hours at room temperature at a flow rate of 150 μ L/min

Our current strategy is to design several thousand of potential signature probes to as many as ten major bacterial groupings at a time and then challenge the arrays with RNA representing each grouping. For example, an array carrying a block of 45mer signature probes for the enteric group as a whole was challenged with *E. coli* 16S rRNA. A large portion of the probes in the enteric block of the array gave a positive signal as predicted. Probes for other nodes populated the surrounding, largely dark (as expected) areas. Probes that gave a poor signal will be excluded from further consideration.

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- Larios-Sanz M, Kourentzi K, Zhang Z, **Willson RC, Pierson DL, Fox GE**. Molecular tools to monitor microbial contaminants during long-term exploration class missions. World Space Congress, 34th Scientific Assembly of the Committee on Space Research (COSPAR) and the 53rd International Astronautical Congress (IAC) of the International Astronautical Federation (IAF), International Academy of Astronautics (IAA), and International Institute of Space Law (IISL). Houston, TX, October 10-19, 2002.
- **Fox GE, Willson RC**. Microorganisms in the spacecraft environment. Bioastronautics Investigators' Workshop, Galveston, TX, January 13-15, 2003.
- Larios-Sanz M, Kourentzi KD, **Fox GE, Willson RC**. Microbial identification using signature probes. American Chemical Society Annual Meeting, New Orleans, LA, March 23-27, 2003.
- Larios-Sanz M, Kourentzi K, Zhang Z, **Willson RC, Pierson DL, Tucker DL, Fox GE**. Molecular tools to monitor microbial ecosystems during long-term exploration class missions. 103rd General Meeting of the American Society for Microbiology, Washington, DC, May 18-22, 2003.
- Tucker DL, Ott CM, Pierson DL, **Willson RC, Fox GE**. Functional genomic analysis of *E. coli* in a low-shear modeled microgravity environment. 103rd General Meeting American Society Microbiology, Washington, DC, May 18-22, 2003.
- **Fox GE, Willson RC**. Signature probes and the design of phylogenetic arrays. Cambridge Healthtech Institute 11th Annual Nucleic Acid-Based Technologies Conference, Baltimore, MD, June 3-4, 2003.

- **Willson RC, Fox GE.** New approaches to nucleic acid purification: Compaction precipitation and metal-chelate affinity. International Symposium on Biorecognition and Affinity Technology, Cambridge, UK, August, 2003.
- **Willson RC.** Nucleic acid purification: New applications and approaches, University of Delaware, Dept. of Chemical Engineering, Newark, Delaware, October, 2003.

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- **Fox GE, Willson RC, Zhang Z.** Methods for determining the genetic affinity of microorganisms and viruses. Submitted 2002.
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Muscle Alterations and Atrophy Team 2003 Report

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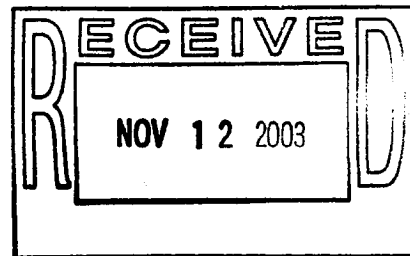
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I. Abstract.

The research mission of the Muscle Alterations and Atrophy Team (MAAT) is to ascertain the underlying mechanisms associated with the loss of muscle mass, strength, and endurance that are the cornerstones of the structural and functional deficits that occur when individuals (human and animal) are subjected to prolonged states of inactivity or skeletal muscle unloading. A key element of this research mission is to, over time, elucidate countermeasures that can effectively ameliorate these deficits using a variety of strategies such as exercise, nutritional and pharmacological interventions, as well as evolving the unique strategy of human powered artificial gravity (assuming that both NASA and the NSBRI becoming truly committed to exploring this modality as a countermeasure strategy). In the past fiscal year significant progress has been made in gaining a better understanding of 1) the factors that induce/affect muscle atrophy, 2) the key genes that are impacted by muscle unloading stimuli, 3) the functional consequences of the atrophy process, and 4) the efficacy of using a) relatively common therapeutic agents and b) a simple isometric resistance training program to significantly reduce the atrophy response in rodent skeletal muscle. This latter observation has the potential to be translated to human experimentation as a potential countermeasure. In the 2003 NSBRI NRA for the skeletal muscle system, a high priority for future research was centered on translational research involving humans in order to bring about a more balanced research portfolio bridging animal and human research.

II. Introduction

In the Fall of 2000, the National Space Biomedical Research Institute's (NSBRI) Muscle Alterations and Atrophy Team (MAAT) began its second three-year funding cycle on research dealing with the structural and functional deficits of the skeletal muscle system that occur in response to prolonged exposure to space flight or the environment of microgravity. Of the eight original projects that were selected in the first period of funding (1997-2000), only one project was selected for continuation. The PI for the project was Dr. Alfred Goldberg, Harvard Medical School; and Dr Ken Baldwin, University of California, Irvine served as a co-investigator on that original project.

In the Fiscal-Year 2000-2003 funding cycle, seven new projects were selected for funding in addition to Dr. Goldberg's project. Two projects were selected for a funding cycle that started on October 1, 2000. These selections involved projects headed by Dr. Baldwin and Dr. Goldberg, respectively, who now serve as the Team Leader and Co-Team Leader for the MAAT. The remaining six projects were recently initiated in funding cycles at different starting dates in the 2001 calendar year. These projects are defined in the next section, Program Structure and Design. During Fiscal Year 2001-2002, two additional projects from the Integrated Function Team were reassigned to the MAAT such that there are now a total of ten research projects that define this team.

The research mission of the MAAT is to ascertain the underlying mechanisms associated with the loss of muscle mass, strength, and endurance that are the cornerstones of the

structural and functional deficits that occur when individuals (human and animal) are subjected to prolonged states of inactivity or skeletal muscle unloading. A key element of this research mission is to, over time, elucidate countermeasures that can effectively ameliorate these deficits using a variety of strategies such as exercise, nutritional and pharmacological interventions, as well as evolving the unique strategy of human powered artificial gravity (assuming that both NASA and the NSBRI becoming truly committed to exploring the modality as a countermeasure strategy).

Muscle Deficits and Critical Concerns to Be Addressed by the Muscle Team.

The following deficits/concerns have been identified in the critical pathway of understanding astronaut health and safety during prolonged spaceflight. These include:

1. Reduced muscle mass (atrophy), which is thought to be due to an imbalance in protein synthetic to protein degradation activity within targeted fibers. The mechanism(s) for such a response is largely unknown.
2. Reduced muscle strength leading to a decrease in physical activity performance and high power output capacity. Deficits in strength often exceed the loss in muscle mass suggesting that more complex mechanisms are responsible for the reduced performance.
3. A slow-to-fast shift in the contractile protein phenotype, e.g., shifts to expression of faster myosin heavy chain (MHC) and calcium cycling proteins. These alterations induce the muscle fibers to become less economical in sustaining force output and locomotor activity.
4. A decreased resistance to fatigue, (which could have functional implications in the performance of extra vehicular activity in space and in performing emergency egress activity upon space craft landing.) This problem is relevant to the other deficits outlined above.
5. A proneness to muscle injury, which is due to the atrophy and loss of strength. An additional outcome of the muscle weakness could cause increased susceptibility to accidents that, in turn, could cause damage to other systems, e.g., bone fractures.
6. Changes in muscle properties are closely linked to changes in the ability of nervous system to accurately control movements; and thus such changes affect safety when performing any type of work.

III. Research Program Structure and Design

Listed below are the research topics and the associated Principal Investigators that form the backbone of the MAAT Strategic Plan. These include:

- Role of Muscle Loading Conditions on Mechanisms of Protein Translation and Their Impact on Unloading-Induced Atrophy -(PI: K. M. Baldwin; University of California, Irvine). This project addresses Concerns #1 and #2.
- The Activation of Protein Breakdown Upon Unloading and Possible Countermeasures—(PI: A.L. Goldberg; Harvard Medical School). This project addresses Concern #1.
- Calpains in Simulated Microgravity-induced Muscle Atrophy -(PI: P. B. Antin; University of Arizona). This project addresses Concern #1.
- Genomics of Human Skeletal Muscle During Bedrest and Exercise --(PI; M. Hamilton; University of Missouri, Columbia). This project addresses Concerns #1-4.
- Gene Expression Profiling of Unloaded Skeletal Muscle -(PI; S. Kandarian: Boston University). This project is also linked to Concerns #1-4
- In Vivo Stress Strain Dynamics in Human Muscle -(PI:S. Sinha; University of California, Los Angeles). This project addresses Concerns #4-6
- .Redux Modulation of Muscle Fatigue and Atrophy Processes in (Simulated) Microgravity -(PI; M. Reid: Baylor College of Medicine). This project addresses Concern # 2 and 4.
- Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle-(PI; P. B. Chase: Florida State University). This project addresses concerns #4-6.
- Calcium Homeostasis and Muscle Phenotype -(PI: R. Wiseman: Michigan State University). This project addresses Concerns #3 and 4.
- Human Muscle Energetics and Mechanics-(PI; M. Kushmerick: University of Washington). This projects addresses Concerns #4-6.

IV. Research Program Accomplishments

Key Findings From the Baldwin Team:

In years one & two, we focused on three specific project areas 1) understanding the underlying processes impacting muscle atrophy process in response to complete muscle inactivity, induced by a novel model of unloading called spinal isolation; 2) characterizing the efficacy of different contraction modes for induce skeletal muscle hypertrophy, which could potentially evolve to a paradigm countermeasure for reducing unloading induced atrophy; and 3) assessing the effectiveness of short term isometric training regimens in blunting the rapid phase of unloading induced atrophy which occurs during the first week of unloading. The following are summaries as to what we accomplished in addressing these three important topics.

Atrophy and Protein Deficits in Response to Muscle Inactivity

The goal of this study was to use the model of spinal cord isolation (SI), which blocks nearly all neuromuscular activity while leaving the motoneuron-muscle fiber connections intact, to characterize the cellular processes linked to marked muscle atrophy. Rats randomly assigned to normal control and SI groups were studied at 0, 2, 4, 8, and 15 days after SI surgery. The slow soleus muscle atrophied by ~50%, with the greatest degree of loss occurring during the first 8 days. Throughout the SI duration, muscle protein concentration was maintained at the control level, while myofibrillar protein concentration steadily decreased between 4 and 15 days of SI, and this was associated with a 50% decrease in myosin heavy chain (MHC) normalized to total protein. Actin relative to the total protein was maintained at the control level. Marked reductions occurred in total RNA and DNA content, and in total MHC and actin mRNA expressed relative to 18S ribosomal RNA. These findings suggest that two key factors contributing to the muscle atrophy in the SI model are 1) a reduction in ribosomal RNA that is consistent with a reduction in protein translational capacity, and 2) insufficient mRNA substrate for translating key sarcomeric proteins comprising the myofibril fraction, such as MHC and actin. In addition, the marked selective depletion of MHC protein in the muscles of SI rats suggests that this protein is more vulnerable to inactivity than actin protein. This selective MHC loss could be a major contributor for the previously reported loss in the functional integrity of SI muscles. Collectively, these data are consistent with the involvement of pretranslational and translational processes in muscle atrophy due to SI.

Molecular Markers of Atrophy and Protein Deficits in Response to Muscle Inactivity

For this project we examined the expression of several molecular markers of protein balance in response to skeletal muscle atrophy induced by spinal cord isolation (SI, i.e., a complete transection of the spinal cord at both a mid-thoracic and a high sacral level plus complete deafferentation between the two transection sites). This treatment nearly eliminates neuromuscular activity (activation and loading) of the hindlimb muscles while maintaining neuromuscular connectivity. SI was associated with a reduced transcriptional activity (via pre-mRNA analyses) of myosin heavy chain (MHC) and actin. In addition, there was an increased gene expression of enzyme systems impacting protein degradation (calpain 1; plus enzymes associated with polyubiquitination processes) that could further contribute to the protein deficits in the SI muscles via degradative pathways. IGF-I receptor and binding protein-5 (BP-5) mRNA expression was induced throughout the 15-day period of SI, while IGF-I mRNA was induced at 8 and 15 days. These responses occurred in the absence of an upregulation of translational regulatory proteins (p70-S6 kinase; 4EBP1) to compensate for the decreased protein translational capacity. These data collectively demonstrate that 1) the molecular changes accompanying SI-induced muscle atrophy are not necessarily the reverse of those occurring during muscle hypertrophy, and 2) the rapid and marked atrophy that defines this model of muscle inactivity is likely the result of multifactorial processes affecting transcription, translation and protein degradation.

Skeletal Muscle Hypertrophy in Response to Isometric, Lengthening and Shortening Training Bouts of Equivalent Duration.

Movements generated by muscle contraction generally include periods of muscle shortening and lengthening as well as force development in the absence of external length changes (isometric). However, in the specific case of resistance exercise training, exercises are often intentionally designed to emphasize one of these modes. The purpose of the current study was to objectively evaluate the relative effectiveness of each training mode for inducing compensatory hypertrophy. Using a rat model with electrically stimulated contractions, groups of rats completed 10 training sessions in 20 days. Within each training session the stimulation duty cycle was equal across the three modes. While this protocol provided equivalent durations of duty cycle, the torque integral for the individual contractions varied markedly with training mode such that: lengthening > isometric > shortening. The results indicate that the hypertrophy response did not track the torque integral with mass increases of: isometric 14%, shortening 12% and lengthening 11%. All three modes of training resulted in similar increases in total muscle DNA and RNA. Muscle mass was highly correlated with the 10-session-mean force integral for isometric and shortening but not lengthening actions. The results of this study indicate that relatively pure movement mode exercises result in similar levels of compensatory hypertrophy that do not necessarily track with the total amount of force generated during each contraction.

Isometric-Mode Exercise As a Countermeasure to Unloading Induced Atrophy

Based on the findings, isometric exercise appears to be as effective as both lengthening- and shortening contractions under high loading conditions. Therefore, we have initiated studies to examine the effectiveness of isometric contractions (4 sets of ten 2-second contractions with 20 second rest intervals between each contraction and 5 minutes of rest between sets) on its ability to reduce the early-onset of muscle atrophy that is a characteristic feature of the hindlimb suspension model. Preliminary findings clearly show that the mixed fibered medial gastroc (MG) muscle weight /normalized to body mass was significantly blunted relative to the contralateral muscle which was unloaded, but not resistance trained. These findings on rats suggest that the simple mode of isometric contraction can be effective in retarding the rapid loss of muscle weight that occurs during the early stages on unloading in which the muscles appear to be the most vulnerable to unloading induced muscle protein degradation.

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Adams, G. R., D. C. Cheng, F. Haddad and **K. M. Baldwin**. Skeletal muscle hypertrophy in response to isometric, lengthening and shortening training bouts of equivalent duration. (Submitted)

Key Findings From the Goldberg Team.

1) We have continued to use transcriptional microarray profiling to identify the set of genes (which we call, atrogins) whose expression is altered when muscles atrophy, independently of the stimulus. (e.g. unloading, glucocorticoids, fasting, cancer). Amongst the unexpected new findings during atrophy were that a) mRNA for only one myofibrillar protein (myosin binding protein H) fell significantly, b) adaptations that promote glucose sparing and gluconeogenesis, c) dramatic increases in expression of metallothionein and genes that favor cap-independent translation, d) decreased expression of many growth-related proteins, and increases in certain transcription factors (e.g. Forkhead family). None of these proteins had been previously implicated in muscle atrophy.

2) The most dramatically induced proteins during atrophy is the ubiquitin-ligase (E3), atrogin-1 (which we had reported in 2001); e.g. its mRNA rises 25-40 fold 3 days after inactivation by denervation, spinal isolation, or hind-limb suspension. Surprisingly, by 1-2 weeks, atrogin1 mRNA falls back to control levels as atrophy slows. Thus there appear to be very different early and late phases of the atrophy process.

3) Related knockout experiments (by Bodine et al, 2001) showed that knockout of this protein or MuRF-1 (a second highly induced ubiquitin ligase) reduces muscle atrophy in response to these signals. Inhibitors that block atrogin or its expression are thus attractive pharmacological targets to block atrophy (as also indicated by our extensive in vitro studies).

4) Using muscle cells (C2C12) in culture, we found that glucocorticoids, which promote atrophy, cause rapid induction of atrogin-1 and MuRF1, but IGF-1, a growth factor that causes muscle hypertrophy blocks atrogin induction. Thus, IGF-1 and insulin cause rapid loss of atrogin mRNA as part of their normal

growth-promoting mechanism.

5) This suppression of atrogen transcription by IGF-1 is mediated by the PI3 kinase - AKT signal transduction pathway, as shown by pharmacological and genetic interventions. Using a reporter gene construct, we have defined key elements of the promoter needed for atrogen induction by glucocorticoids and suppression by IGF-1.

6) We have developed a new simple, reversible invitro model for muscle atrophy. Nutrient deprivation of C2C12 myotubes causes a rapid loss of myotube mass, and a dramatic induction of atrogen & MuRF1, which can be prevented by activation of the PI3kinase-AKT pathway. This system should allow efficient screening for possible pharmacological or endocrine countermeasures to retard atrophy.

Publications:

Jagoe, RT, Lecker, SL, Gomes, M, and Goldberg, AL. Patterns of gene expression in atrophying skeletal muscles: the response to food deprivation. *FASEB Journal* 2002, 16: 1697-1712.

Lecker, SL, and Goldberg, AL. Slowing Muscle Atrophy: Putting the Brakes on Protein Breakdown. *J. Physiol.*, 2002 (In Press).

Key Findings From the Antin Team:

The long-term goal this project is to understand mechanisms regulating protein turnover in normal muscles, and to develop methodologies for modulating muscle protein homeostasis in both normal and abnormal conditions. Studies are designed to investigate the ability of increased calpastatin expression to reduce or inhibit muscle atrophy in the mouse hindlimb unweighting model. Towards this goal, we have developed a tetracycline inducible gene expression system in transgenic mice that allows for regulated induction of gene expression specifically in the skeletal muscles of mice. This system involves generating two lines of transgenic mice. The first line, called the transactivator, contains a codon optimized reverse tetracycline transactivator (rtTA) cDNA under control of a skeletal muscle-specific version of the mouse MCK promoter. Transgenic mice containing this construct express rtTA almost exclusively in skeletal muscles. These mice can be crossed to a second transgenic line containing a bi-directional promoter centered on a tet responder element driving both a luciferase reporter gene and a tagged gene of interest; in this case the calpain inhibitor calpastatin. After crossing these lines, mice carrying both transgenes show Doxycycline inducible expression of calpastatin and the luciferase reporter gene. Analysis of double transgenic mice showed that luciferase activities were often induced to more than 10,000 fold following administration of Doxycycline. Calpastatin expression was induced to similar levels. Studies are underway to assess the effects of calpastatin over expression on muscle atrophy using the hindlimb

unweighting model.

Publications:

Grill, M.G., Bales, M.S., Fought, A.N., Rosburg, K.C., Munger, S.J., and Antin, P.B. (2003) Tetracycline-inducible system for regulation of skeletal muscle-specific gene expression in transgenic mice. Transgenic Res: 12: 33-43.

Key Finding From the Sinha Team.

1. A completely non-invasive method was developed and perfected using safe and painless, velocity encoded, phase contrast MR imaging techniques, to measure the effect of muscle atrophy in the human lower leg. The parameters that could be measured and quantified included total volume of different muscle compartments, the maximum torque that the subject could exert and finally, and most importantly, the contraction velocity at any arbitrary point in leg, within any muscle group during an isometric contraction cycle. All three parameters are affected by muscle atrophy.
2. The above methodology was first applied to 10 normal subjects, in whom, atrophy was induced by suspending one leg for a period of 4 weeks. The above method was very effective in showing consistent patterns of changes in all three parameters, muscle volume, force of MVC and the contraction velocity. The method could detect differential changes in different muscles groups, as well as track the return to normalcy as the subjects were given physical rehabilitation/therapy.
3. Similar changes in muscle function (contraction velocity), muscle volume and strength could be quantified and tracked in 3 patients who had undergone surgery to repair Achilles tendon rupture, with consequential muscle atrophy. These changes were further tracked during six weeks of re-ambulation and physical therapy. Once again, consistent patterns of changes could be detected in all three parameters.
4. The structure of human multipennate soleus muscle in vivo as elucidated by high resolution MRI and 3D volume rendering, was correlated with the heterogeneous spatial distribution of functionality, determined in terms of peak shortening velocity during isometric contraction using the above method.

II. Implications for the welfare of Astronauts in Space Environment: One of the most significant problems facing astronauts is that of muscle atrophy resulting from micro-gravity or immobilization during space flights. Methods that can reveal the functional status of different muscle groups, in a robust, clinical and preferably non-invasive and safe manner, could have a significant clinical impact in the physical well being of the astronaut. Knowledge about how the strain distribution, muscle volume and the maximum force the subject can exert, change as atrophied muscle progresses towards normalcy can be a valuable clinical parameter to quantify the relative efficacy of different therapeutic strategies for rehabilitation used for astronauts, and can help one understand the complex interactions between active contractile tissues and passive connective ones.

The method we have developed, using phase contrast, velocity encoded MR imaging technique is such a non-invasive, non-ionizing and safe method that can be used routinely in the clinic. We have been able to prove its efficacy in monitoring the changes after onset of atrophy and the method is sensitive enough to follow the changes as the muscles return to normalcy as the patient is given rehabilitative physio-therapy.

III. Publications:

Published:

Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. *J Appl Physiol.* 2003 Aug;95(2):829-37. Epub 2003 Apr 25.

Accepted:

Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Watching muscles, aponeuroses and tendons move – a 3-D view of the soleus anatomy and function. Accepted by *J Appl. Physiol.*, June, 2003 (#-00596-20030).

Submitted:

S.Sinha, Hodgson JA, Finni T, Lai AM, Edgerton VR, Grinstead J, V. Reggie Edgerton. A Comparison of Phase Contrast and Spin Tagging Methods for Determining Differences in Muscle Kinematics between Normal and Atrophied Muscles. Submitted July, 2003 to *J. Mag. Res. Imag.*

Abstracts:

- S.Sinha, *A.Lai, J.Hodgson, V.R.Edgerton. In Vivo Measurement of Strain Dynamics of the Triceps Surae Muscles-Tendon Complex Using MR Phase Contrast Imaging. Accepted for presentation at 88th Mtng of RSNA, Chicago, 2001.
- S.Sinha, *A.Lai, J.Hodgson, J.Grinstead, V.R.Edgerton. Quantification of Strain Distribution and Volume Changes in Muscle as a result of Atrophy induced by Unilateral Limb Suspension—a Phase Contrast MR study. Accepted to 10th Intl. Soc Mag Res Med Mtng. 2002, Hawaii.
- S.Sinha, J.A.Hodgson, Finni T, *A.Lai, J.Grinstead, V.Reggie Edgerton. Comparison of Muscle Kinematics Quantification Using Phase-Contrast and Spin Tagging Methods of Visualizing Motion. Accepted to 10th Intl. Soc Mag Res Med Mtng. 2002, Hawaii.
- Lai AM, Hodgson JA, Finni T, Edgerton VR, Sinha S. MRI Measurement of In Vivo Behavior of Human Triceps Surae Muscle During Voluntary Submaximal Isometric Contractions. *Amer. Coll. of Sports Medicine, San Fransisco, 2003.*
- Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Non-uniformity of structure and function within the triceps-surae muscle tendon unit. *Amer. Coll. of Sports Medicine, San Fransisco, 2003.*
- Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Triceps Surae Muscle-Tendon Function in Isometric and Passive Movements. 7th IOC Olympic World Congress on Sport Siences, Athens, Oct. 2003.
- Sinha S, Lai AM, Hodgson JA, Finni T, Edgerton VR. Alterations in Volume, Strength and Contraction Velocities of the Triceps Surae Complex after Four Weeks of Lower Limb Suspension and Subsequent Rehabilitation. Accepted for presentation at Radiological Soc. Of N. Amer., Chicago, 2003.
- Sinha S, Hodgson JA, Finni T, Edgerton VR. Correlating Function of the Human Soleus Muscle as Characterized by Peak Velocity during Isometric Contraction, with its Structure. Accepted for presentation at Radiological Soc. Of N. Amer., Chicago, 2003.

Sinha S, Lai AM, Finni T, Hodgson JA, Edgerton VR. Assessment of Functional Recovery after Surgical Repair of Achilles Tendon Rupture from Estimates of Contraction Velocity, Muscle Volume and MVC. Accepted for presentation at Radiological Soc. Of N. Amer., Chicago, 2003.

Key Findings From the Kandarian Team.

The results from the initial phases of the project have recently been published in their entirety (Stevenson 2003). In brief, expression of 309 known genes was significantly changed by at least 2-fold (212 upregulated, 97 downregulated). *K*-means clustering was used to divide these genes into co-regulated clusters based on the similarity of temporal expression patterns. This allowed the development of a timeline of the atrophy process with respect to the behavior of genes in a broad array of functional categories.

Regulatory genes were often upregulated early, in either a transient or sustained manner, but they also populated clusters with later patterns of activation, suggesting different phases of molecular adaptations. Other early events were the activation of ubiquitination genes and downregulation of protein chaperones. In comparison, clusters representing slightly delayed activation patterns included genes involved in fast contraction, glycolysis, translational inhibition, oxidative stress, protein degradation, and amino acid catabolism. Downregulated genes exhibited fewer unique expression patterns and included structural and regulatory genes of the extracellular matrix and cytoskeleton, and genes that define a slow-oxidative phenotype. Other novel findings include the tight co-activation of proteasome subunit and ubiquitination genes, differential regulation of serine proteases and serine protease inhibitors, and the identification of transcriptional, signaling, growth and cell cycle genes that probably play a role in the atrophy process. This work has uncovered temporal patterns of gene expression that highlight the molecular processes that underlie muscle atrophy and provide new avenues for study.

The data summarized above suggest several pathways that are at work during muscle atrophy. These include the activation of proteolytic systems, the inhibition of translation, oxidative stress, extensive remodeling of the extracellular matrix and cytoskeleton, and the activation of several signaling pathways including the JAK-STAT pathway, notch signaling, cytokine signaling, and myogenic signaling. The results also indicate that there are several different temporal switches of regulatory genes that are activated during atrophy. That is, not all transcription factors and signaling factors are up or down regulated early, and not all of them have the same pattern. Some are transient and some are sustained. Also, one of the original aims of this project was to identify specific genes that may be involved in the regulation of muscle protein loss during atrophy. We have carried out some focused study on the Nedd4 gene and this work is providing us with a better understanding of this ubiquitin ligase in proteolysis during muscle atrophy.

Nedd4 project: Results from several laboratories have shown that the ubiquitin-proteasome system is responsible for the majority of muscle protein loss that occurs with disuse. Ubiquitin-protein ligases (E3s) are responsible for the targeting of specific proteins for degradation by the proteasome. Our microarray data reconfirm that Atrogin1 and MuRF1 are upregulated during unloading, but we have also identified another upregulated ubiquitin-protein ligase not previously characterized with respect to muscle atrophy called Nedd4. Nedd4 has a pattern of activation very similar to that of Atrogin1/MAFbx. We have confirmed this pattern of activation at the mRNA and protein

level. Nedd4 is known to ubiquitinate membrane proteins but its role in muscle protein turnover has not yet been defined. We are in the process investigating the role of Nedd4 in muscle using genetic overexpression approaches.

PUBLICATIONS

Kandarian, S.C. and E.J. Stevenson. Molecular events in skeletal muscle during disuse atrophy. *Exerc. Sport Sci Rev.* 30(3):111-6, 2002.

Stevenson, E.J., P.G. Giresi, A. Koncarevic, and S.C. **Kandarian**. Global analysis of gene expression patterns during disuse atrophy in rat skeletal muscle. *J. Physiol.* 551.1, 33-48, 2003.

ABSTRACTS

Koncarevic, A., R. Jackman, **S. Kandarian**. Upregulation of ubiquitin-protein ligase Nedd4 in muscle disuse atrophy. Molecular Biology of Muscle Development and Regeneration. The Banff Centre, Alberta, Canada, June 2, 2003.

Stevenson, E., P. Giresi, A. Koncarevic, **S. Kandarian**. Temporal analysis of muscle gene expression patterns during disuse muscle atrophy. Molecular Biology of Muscle Development and Regeneration. The Banff Centre, Alberta, Canada, June 2, 2003.

Key Findings From the Bryant Team.

The overall goal of this project is to produce a muscle cell model (digital muscle cell) that will: explain biomechanical adaptations that occur with alterations in muscle protein isoforms due to changes in activity level; predict bioenergetic changes associated with changes in activity level; be integrated into computational models of human limb and heart. To accomplish our goal of constructing a digital muscle cell, we will: (1) identify contractile protein composition of skeletal and cardiac muscles from high- and low-activity rats; (2) characterize contractile properties (phenotype) of selected muscles containing unique mixtures of protein isoforms, as identified in Aim 1; and (3) in parallel with Aims 1 & 2, develop the “digital” cell biomechanical model.

We have made significant progress looking at myosin isoform dependence of ATP hydrolysis product inhibition of cellular biomechanics. To correctly model the interaction between cellular energetics and biomechanics (chemo-mechanical transduction) in muscles with heterogeneous fiber populations we measured the product inhibition (inorganic phosphate, Pi) of force development in chemically demembrated single muscle cells from rat and rabbit muscle. Increased cellular levels of Pi are implicated as a mechanism of fatigue in both high intensity and chronic exercise. The relationship between [Pi] and maximum force production was determined in muscle fibers from five different muscles of rat: adductor mangus, gracilis, TFL, psoas and soleus muscles. We also measured this relationship in rabbit psoas and soleus muscle fibers to investigate possible species differences in muscle fiber chemo-mechanical properties. Muscle fiber myosin isoform was determined by gel analysis. Results from these studies indicate differences in the Pi-sensitivity of force between different muscle

types and between similar muscles of different species. The data were modeled with a kinetic model of chemo-mechanical transduction for muscle actomyosin crossbridges that is based on mass action principles. This model could successfully predict the Pi-force relationships for all the muscle fiber types used in experiments. Importantly, the predictions of the model were sensitive enough to determine the effects of accumulating Pi at the levels likely to be seen during fatiguing exercise. This supports the utility of this model as a valid component of the digital cell in predicting functional alterations that occur with alterations in muscle use.

- Implemented an animal model to compare low activity with high voluntary activity rats
- Identified myosin binding protein C (C-protein) isoform differences between soleus muscles from low versus high activity rats
- Inorganic phosphate (Pi) decreased force of fast and slow muscles over the temperature range 10 - 35°C; as temperature increases, force increases and the inhibitory effect of Pi decreases
- Developed a computational, molecular level model for muscle that incorporates filament compliance and Ca²⁺ regulation; modeled force and ATP consumption for individual twitches of fast and slow muscles; demonstrated that filament compliance could modulate steady-state activation of force

Manuscript in preparation:

Thomas L. Daniel, Michael Macpherson and P. Bryant Chase. A biochemical model of the muscle half sarcomere: effect of myofilament compliance on calcium activation. (In Preparation)

Abstracts

P. Bryant Chase, Michael Macpherson and Thomas L. Daniel. 2002. A spatially explicit, 3-D model of the muscle sarcomere. *Biophys. J.* 82:5a.

Jennifer Fredlund, Michael Regnier and P. Bryant Chase. 2003. Inorganic phosphate (Pi) dependence of force in fast and slow fibers from different rat and rabbit skeletal muscles. *Biophys. J.* 84:450a.

Bertrand C. W. Tanner, Michael Regnier, P. Bryant Chase and Thomas L. Daniel. 2004. Modeling different nearest-neighbor interactions with a spatially explicit, calcium-regulated, compliant myofilament model. *Biophys. J.* 86: in press.

Aya Kataoka, Brenda Schoffstall, Amanda Clark and P. Bryant Chase. 2004. Effect of rapamycin on skinned skeletal muscle fiber force. *Biophys. J.* 86: in press.

Presentations (no published abstract)

“Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle,” NSBRI Integrated Human Function Core Workshop, San Diego, CA (April, 2001)

“Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle,” NSBRI Investigators Meeting, Houston, TX (January, 2002)

“Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle,” NSBRI Integrated Human Function Core Workshop, Seattle, WA (August, 2002)

“Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle,” Bioastronautics Investigators’ Workshop, Galveston, TX (January, 2003)

Key Findings From the Reid Team.

Exercise-induced fatigue and muscle atrophy are mediated in part by reactive oxygen species (ROS), a stimulus that may be exaggerated by radiation during spaceflight. The current project is assessing the roles of ROS signaling and radiation on muscle fatigue and atrophy and is testing antioxidants as possible countermeasures. Progress was hindered by severe damage to our institution by Tropical Storm Allison in June, 2001. These losses were resolved and we have made rapid progress on the project over the last year as outlined below:

Aim 1. To determine if oxidative stress contributes to muscle fatigue during handgrip exercise. Fatigue of hand and forearm muscles may limit crew performance during extravehicular activity (EVA). N-acetylcysteine (NAC) is an antioxidant that inhibits muscle fatigue in humans. We have recently completed experiments testing the capacity of NAC to inhibit muscle fatigue and oxidative stress in humans during handgrip exercise. Working with Dr. Jeff Jones, Flight Surgeon at NASA Johnson Space Center, we used equipment and test procedures designed for use on the International Space Station. Results of the study show the feasibility of this approach. NAC abolished glutathione oxidation blood draining the affected muscle groups and increased handgrip endurance by 30% relative to untreated trials. This aim has been completed and a manuscript is being prepared. Follow-up studies are being planned that will test the importance of these findings in a more operationally-relevant setting.

Aim 2. To determine whether ionizing radiation accelerates ROS production and fatigue in skeletal muscle. We postulated that proton radiation absorbed during EVA would increase tissue ROS levels and accelerate muscle fatigue. We were testing this postulate in collaboration with Dr. Carlos Gonzalez, Director of the cyclotron at the University of Texas Medical School, when Tropical Storm Allison destroyed the cyclotron facility in 2001. The facility has not been rebuilt. Resources intended for these experiments have been redirected to studies of mechanisms regulating muscle atrophy and to tests of potential countermeasures (Aim 3).

Aim 3. To evaluate oxidative stress as a mediator of muscle weakness caused by gravitational unloading. Muscle atrophy and contractile dysfunction cause weakness after prolonged spaceflight. We are evaluating oxidative stress as a cause of these changes in mouse soleus during 12-days of hindlimb unloading and are using cell culture

techniques to evaluate mechanism. Our results show that: 1.) unloading increases oxidant activity within soleus muscle fibers; 2.) contractile dysfunction is blunted by administration of some antioxidants (NAC, allopurinol) but not others (curcumin, vitamin E); 3.) a novel ubiquitin conjugating enzyme, UbcH2/E2_{20k}, is highly expressed in skeletal muscle, is upregulated by ROS exposure, and mediates ubiquitin conjugation to muscle proteins; 4.) hydrogen peroxide upregulates expression of atrogin1/MAFbx, a key ubiquitin ligase that regulates muscle atrophy; 5.) this signal is transduced by p38 MAP kinase; and 6.) p38 inhibition blocks atrogin1/MAFbx upregulation and the associated rise in ubiquitin conjugating activity. In Year 3, we will test the roles of these transcriptional mechanisms in atrophy of unloaded muscle and will continue to evaluate potential countermeasures.

Aim 4. To determine if radiation stimulates atrophic signaling in muscle We postulated that radiation-derived ROS might stimulate catabolic signaling and planned to measure activity of redox-sensitive pathways in muscle after proton irradiation. Destruction of the Medical Center cyclotron has prevented these experiments. Project resources have been redirected to studies of cellular mechanism and putative countermeasures (Aim 3).

Publications:

Li, Y.-P., S.M. Lecker, Y. Chen, A.L. Goldberg, and M.B. Reid. TNF- α increases ubiquitin conjugating activity in skeletal muscle by upregulating UbcH2/E2_{20k}. *FASEB J.* 17: 1048-1057, 2003.

Li, Y.-P., Y. Chen, A. Li, and M.B. Reid. Hydrogen peroxide stimulates ubiquitin-conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes. *Am J Physiol Cell Physiol.* 285(4):C806-C812, 2003.

Submitted

Durham, W.J., Y.-P. Li, E. Gerken, M. Farid, R.R. Wolfe, and M.B. Reid. Fatiguing exercise reduces DNA-binding activity of NF- κ B in skeletal muscle nuclei. *J Appl Physiol.* In review.

In Preparation

Matuszczak, Y., M. Ferid, J. Jones, A.A. Taylor, and M.B. Reid. N-acetylcysteine effects on glutathione oxidation and endurance during handgrip exercise. *J Appl Physiol* In preparation.

Matuszczak, Y., S. Arbogast, M.B. Reid. Allopurinol mitigates contractile dysfunction caused by unloading of murine hindlimb muscle. *J Appl Physiol* In preparation.

Li, Y.-P., J. Sacheck, Y. Chen, J. John, A.L. Goldberg, and M.B. Reid. TNF- α and H₂O₂ act via p38 MAPK to upregulate expression of the ubiquitin ligase atrogin1/MAFbx. *J Biol Chem* In preparation.

Farid M., W.J. Durham, and M.B. Reid. Effects of dietary curcumin on NF- κ B activity and contractile performance in ambulatory and unloaded murine soleus. *J Nutr* In preparation.

Gerken, E., and M.B. Reid. N-acetylcysteine blunts the contractile dysfunction induced by unloading of mouse antigravity muscles. *Am J Physiol: Cell Physiol* In preparation.

Durham, W.B., Y.-P. Li, F. Mehran, and M.B. Reid. Catabolic signaling in antigravity muscles of mice subjected to chronic unloading. *J Appl Physiol* In preparation

Key Findings From the Hamilton Team.:

1. Translational studies using humans and rodents are providing an unprecedented understanding of the genes underlying muscle alterations to unloading of skeletal muscle.

- In describing the “human skeletal muscle unloading genome”: ~300 changes in gene expression have been identified. This includes dozens of genes that are very reproducibly influenced by unloading in humans, but have never been studied before by muscle researchers.
- This work is important because there is a consensus that the field is currently in a stage that needs genuine translational projects that build a two-way street for the mechanistic and applied developments to progress synergistically.
- *A priori* evidence of the most robust changes in gene expression in human muscles during unloading will help guide ongoing and future studies using *rodent* models. Thus, we are enhancing these translational insights by publishing our databank that directly compares which of the human transcriptional responses are known to be mimicked in the rat hindlimb unloading model.
- Using the rat hindlimb unloading model, we recently reported the set of genes most sensitive to even very short periods of unloading and reloading. *Over 100 new candidate genes for muscle alterations were published* that used very conservative criteria. *This study was important in part because it has identified transcriptional responses that could act as the initial triggers to muscle adaptations, before secondary responses due to muscle atrophy and alterations occur.*
- *How does this relate to welfare of astronauts in the space environment?* 1) As with most every major medical breakthrough, animal work will play a large role. However, the key to our work is that we are *helping to make sure the rodent research that will be done, will be geared toward making new advances in altering the transcriptional processes that occur in human muscle during unloading.* 2) If one looks at modern medical research in general, almost every field is major historical strides by using microarray transcriptional research, and space medicine should be no exception. Space exploration is limited in part by diverse skeletal muscle problems (see below), and if we are to solve this problem, knowing the transcriptional reasons that underpin the causes for the problems is clearly essential to develop the ideal countermeasures. Sections 6.1, 6.2, and 6.6 of the NSBRI Muscle Alterations and Atrophy Strategic Plan describe goals and research needs we have been working to address. This

provides a cogent vision that has evolved over many years by muscle researchers as to what are the skeletal muscle problems as defined in the Critical Path Roadmap, and 7 major NSBRI goals that need to be kept in mind to solve the problems.

II, We identified and published regarding a transcriptionally-dependent process in skeletal muscle was rapidly invoked during unloading that repressed lipid metabolism.

How does this relate to welfare of astronauts in the space environment or to the welfare of others? Findings have great relevance to unloading conditions like space flight, but also relevance to the broader problems *on earth during physical inactivity* (NSBRI goal #6). Owing to its large mass, skeletal muscle metabolism has *integrative effects* that influence the metabolism of other tissues and the overall health of the individual.

- We have a paper coming out in publication in September of 2003 that shows that *Virtually all (90-95%) of the skeletal muscle-specific lipoprotein lipase activity was inhibited by unloading.* We showed in rats that the process was dependent upon a local transcriptional mechanism only *in muscle* and not other tissue. The physiological consequences included a 75% reduction of the clearance of radiolabeled plasma triglyceride rich lipoprotein by skeletal muscle. As a secondary consequence, high density lipoprotein cholesterol was also reduced significantly by 22%.
- One implication for this is that now we have learned that long-term space flight, or similar conditions on earth, will be impacted by skeletal muscle lipid metabolism. One potential positive countermeasure that we reported in our publication was that an acute bout of moderate exercise was capable of preventing 100% of the bad effects of unloading on lipid metabolism.
- A second implication is that recent progress by others has shown the lipid content of myocytes has profound effects on signaling for alterations in some muscle alterations.

III. *Studies have begun to systematically develop exercise countermeasures that are feasible and provide optimal protection against diverse muscle alterations.*

- We found in ongoing studies using a straightforward program involving local contractions (one-leg exercise), that combines both endurance and resistance training which could be performed in a microgravity environment, was effective at preventing ~72% of the changes in gene expression.
- The most positive physiological consequence included slightly more than a 3-fold reduction in the time to fatigue when performing a local muscle endurance test to evaluate the ability to perform work after 17 days of unloading.
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Publications.

Bey, L, N. Akunuri, P. E. Hoffman, P. Zhao, D.G. Hamilton, and **M.T. Hamilton.** Patterns in global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activity. *Physiol Genomics* 16;13(2):157-67, 2003.

Bey, L. and **M.T. Hamilton**. A molecular reason to maintain daily low-intensity activity: Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity. *J. Physiol. (London)*, 551.2: 673-682, 2003.

Key Findings From the Wiseman Team.

In the Past Year (Year 01) our research goals were two-fold: First, to understand the role of ATP homeostasis in altering calcium handling properties of muscle cells, and secondly, to evaluate the transcriptional responses to the resulting calcium signaling patterns. In the first year, we have successfully identified and characterized an energetically challenged model to being testing our hypotheses. The progress in defining the energetics is described briefly in the following paragraphs. Calcium measurements are currently ongoing in both wild type and our model and will be completed before subsequent goals relating these changes to transcription factor responses can begin.

We explored the energetics of a known genetic defect in ATP handling, a transgenic mouse deficient in creatine kinase, a promising way to perturb the early events of calcium and ATP homeostasis. This enzyme is important in initial events of nucleotide handling (ADP buffering) and also potentially in calcium handling because the kinetics of sarcoplasmic reticular ATPase activity (calcium pumping) depends critically on adenine nucleotides. The results show that while there was no difference in resting PCr/ATP ratio in control vs. MMKO muscles the relative PCr changes after 2 s bursts of contractions at 5 Hz is quite dramatic (Figure 1) where the initial rate of PCr hydrolysis was decreased by over 75% in MMKO vs. control muscles. Concurrently peak twitch force after 2 s of 5 Hz stimulation was 95.6 ± 1.6 % of initial in control muscles vs. 70.9 ± 1.9 % in MMKO muscles thereafter the steady state approached a similar level (Fig. 2). The increase in estimated ADP associated with this deficit may explain the rapid decrease in twitch force observed at the onset of stimulation in MMKO muscle. This dramatic increase in ADP might alter calcium sequestration and/or release, thereby accounting for the rapid, compensating decrease in twitch force in MMKO muscles. In ongoing experiments we are investigating the changes in calcium homeostasis in fast-twitch, slow-twitch and mixed fiber phenotypes. Further, we have also commenced work on our second goal, to understand how muscles transcriptionally respond to these physiologic challenges.

- 1- Large perturbations in energetics through transgenic manipulations cause transient, but severe mechanical limitations due to creatine kinase deletion. Large increases in free ADP were measured. These energetic changes are being correlated with altered calcium homeostasis in isolated muscles. This is currently under investigation in this mouse line.
- 2- Direct inhibition of creatine kinase in wild type mouse muscles causes dysfunction in calcium handling. Studies are underway to measure the extent of calcium handling changes in parallel with work in #1.
- 3- Unilateral limb electrical stimulation model developed for these studies shows increased mRNA expression of contractile and metabolic proteins consistent with fast to slow muscle phenotype transformation. Pharmacologic manipulations to modulate calcium homeostasis cause further increases of mRNA expression for these genes. This work is being prepared for publication.

4- Modulating calcium homeostasis in vivo with a ryanodine receptor activator causes large increases in expression of mRNA for slow muscle in fast muscle phenotypes. This suggests a potential target for pharmacologic countermeasure development. This work is being prepared for publication.

5- Gene array data for unilateral leg stimulation shows increases in proteins for contractile proteins, calcium handling, metabolism, transcriptional control and apoptosis. Ryanodine receptor activation causes elevation of these genes but also an additional group of genes related to proliferation and differentiation. This work is near completion.

Publications

Roman, BR, RA Meyer and RW Wiseman. 2002. Phosphocreatine kinetics at the onset of contractions in skeletal muscle of MM creatine kinase knockout mice. *Am. J. Physiol.* 283(6):C1776-83.

Christopher Ontiveros, Regina Irwin, Robert W. Wiseman and Laura R McCabe. 2003. Hypoxia suppresses runx2 independent of modeled microgravity. *Journal of Bone and Mineral Research* (submitted).

3) Ronald A. Meyer, Theodore F. Towse, Robert W. Reid, Roop C. Jayaraman, R.W. Wiseman, and Kevin K. McCully. 2003. BOLD MRI mapping of transient hyperemia in skeletal muscle after single contractions. *NMR in Biomedicine*. (submitted).

Meyer, RA and RW Wiseman. 2003. The metabolic systems: control of energy metabolism in skeletal muscle. *Graduate Textbook in Exercise Physiology*. Ed. C. Tipton. Elsevier. (submitted).

Abstracts:

1) Ontiveros C, R Irwin, RW Wiseman and LR McCabe. 2003. Hypoxic Conditions of the RWV decreases RUNX2 and osteoblasts differentiation. *J Bone Min Res.* 18: S343.

2) DP Ankrapp*, RC Jayaraman*, JN Dentel, ER Wehrwein and RW Wiseman. 2003. The effects of electrical stimulation and caffeine administration on slow muscle mRNA expression in mouse fast-twitch muscles. *Experimental Biology Meeting*, San Diego, CA.

3) Reid, R, T. Towse, R Wiseman and R. Meyer. 2003. Brain activation during muscle recruitment. *Int. Soc. Mag. Res. Med.* Toronto, Canada.

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Key Findings from the Kushmerick Team

There is good progress to report on all aspects of the project: human muscle energy metabolism; intramuscular blood flow; mathematical modeling of muscle energy metabolism.

Comparisons among 6 subjects concerning their oxidative phosphorylation flux capacity with the fraction of that capacity used in a standard series of 2 min isometric twitches as a standard stress test demonstrates that there is significant variation in both parameters. The significance of this is that different normal subjects have different economy (high ATPase for the same mechanical output) and different capacity for ATP generation. For example, one subject on one extreme had a high ATPase and low oxidative capacity vs subject on the other end of the range with the opposite response. These experiments were made on the tibialis anterior muscle in the leg.

Dynamic working contractions cost more energy than isometric ones, when compared by comparable parameters, e.g., the tension-time integral during the contraction. The main point is that it is possible to do these classical energetic experiments in human muscle, and it will be possible to get a good measure of human muscle efficiency, and test the hypothesis that different individuals have different intrinsic efficiency (work output per unit chemical energy input).

Out current work is testing reproducibility and quantifiability, as well as the linearity of the graded response to graded exercise, and the time course of the post-exercise hyperemic response as compared with the known time course of oxidative phosphorylation measured by NMR spectroscopy.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL TEAM REPORT November 3, 2003

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I. ABSTRACT

The NSBRI Neurobehavioral and Psychosocial Factors Team encompasses the Critical Path Roadmap scientific questions from two of the four areas subsumed under Behavior and Human Performance: (1) Human performance failure because of poor psychosocial adaptation (area 18), and Human performance failure because of neurobehavioral dysfunction (area 21). The Team is charged with conducting research for the development of countermeasures that will ensure that astronaut behavioral health is maintained during prolonged space missions, and that both individual astronaut and crew functioning are effectively optimized. There are eight ground-based projects making up the current Team. The studies are divided equally between psychosocial and neurobehavioral scientific questions. They cover a considerable breadth of scientific techniques—projects range from studies of humans living in analog environments (Antarctica, Mount Everest) to laboratory studies of small teams performing on synthetic task environments, to neurobehavioral studies of performing under conditions of stress. This breadth notwithstanding, the overarching focus of all eight projects is on the impact of stress—individual and interpersonal—on behavioral functions. Collectively, the projects seek to identify (1) the causes of stress, and its consequences for astronaut cognitive, affective and social functioning; (2) techniques to objectively detect stress reactions and performance deficits in individuals and groups in the remoteness of space; and (3) countermeasures to prevent and otherwise mitigate the occurrence of stress reactions and their adverse effects on individual and crew performance. During the past year all projects have completed or nearly completed data acquisition, and progressed to data reduction and hypothesis testing. Project 1 (Wood et al.) is studying the role of personality, culture, and group characteristics on both individual and group performance in Antarctica. Measures of personality, performance, psychological responses and biological markers of stress have been obtained on N=100 wintering-over volunteers. One important finding to emerge from preliminary analyses was that women were more adversely affected than men relative to the effects of poor team cohesion on individual motivation to work. Project 2 (Brady et al.) and Project 3 (Orasanu et al.) have gathered extensive data on different synthetic task environments, which provide realistic simulations of small-group performance, communication and problem-solving of the kinds required in space flight. These projects are documenting ways to counter the negative impact on team performance from intra-team conflict and stressful conditions. Project 4 (Carter et al.) has completed consultation interviews with more than a dozen astronauts who have long-duration space flight experience and numerous ground control personnel, to develop the content and design of the conflict resolution and depression sections needed for a prototype system. The conflict resolution and depression modules include training content on how to prevent, assess, and manage these problems. Project 5 (Dinges et al.) has completed data acquisition on performance-induced workload stress in N=60 volunteers, in order to evaluate a novel computer recognition algorithm for unobtrusive 3-dimensional tracking of facial expression. The approach allows the translation of 2D video footage to a form that can be used for tracking the 3D orientation and translation of the face. It is being tested and improved to provide an unobtrusive technique for monitoring stress during performance onboard long-duration spacecraft. Project 6 (Lieberman et al.) has also made significant progress in developing and validating an unobtrusive speech-based computer algorithm for detecting cognitive impairment associated with hypoxia and stress in Everest climbers. Two speech measures showed promising results. Project 7 (Kosslyn et al.) has completed development of the MiniCog, which is a set of brief cognitive performance tasks on a hand-held device that can be used to quickly assess cognitive capability in remote locations. Validation is underway. Project 8 (Aston-Jones et al.) has established that low-dose clonidine may be an effective treatment for stress-induced performance deficits in an animal model. It is anticipated that all projects will complete hypothesis testing during the next few months.

II. INTRODUCTION

The Neurobehavioral and Psychosocial Factors Team was formed in 2000, and funded in 2001, following peer-review of applications for research to mitigate risks to both individual behavioral health (i.e., neurobehavioral functions) and group processes (i.e., psychosocial functions) during space flight. Specifically, the Team seeks to counter the development of psychosocial risks manifested through inadequate leadership; interpersonal strife or social alienation (e.g., due to gender, culture or status differences); poor group teamwork; lack of crew coordination in problem solving; ineffective communications within the team or with ground controllers; and loss of crew morale. In parallel, other projects on the Team seek to counter the development of neurobehavioral problems manifested through stress reactions; anxiety; depression; loneliness; anger; and neurocognitive impairments.

Unlike some areas of NSBRI research, where there is a single source for the biomedical problem (e.g., microgravity effects on muscle or bone), there are a considerable number of factors in prolonged space flight that could create or contribute to neurobehavioral and psychosocial dysfunctions (e.g., excessively scheduled activities and work requirements, poor physiological adaptation to microgravity; interpersonal strife; perceived risks to health; loneliness for family; inadequate communication with Earth; habitability constraints; radiation). Consequently, the countermeasures being developed through the research by the Neurobehavioral and Psychosocial Factors Team necessarily must cover an array of issues and approaches. The following are the various categories in which countermeasure development is anticipated from the research on the Team.

- 1—Selection criteria for optimal crew cohesion, including culture and gender diversity
- 2—Training for group living; training for flight and ground crew optimal relations
- 3—Guidelines to optimize communication for crew decisions and problem solving
- 4—Technologies for monitoring and early diagnosis of cognitive problems and emotional distress
- 5—Behavioral treatments for stress; affective disorders; and for resolving team conflicts
- 6—Pharmacological treatments for stress; affective disorders and serious neuropsychiatric and neurological reactions
- 7—Habitability strategies for privacy; and work strategies for motivation and performance
- 8—Support for relaxation and leisure activity for enhancing quality of life
- 9—Support for assimilating crews psychosocially and neurobehaviorally after return
- 10—Novel countermeasure opportunities identified by NASA and through new scientific efforts
- 11—Development of a database on the neurobehavioral and psychosocial effects of countermeasures for other biomedical problems in space flight

III. TEAM STRUCTURE AND DESIGN

Ideally, the risks to individual and group behavioral health created by spaceflight are best dealt with through prevention (e.g., a well-integrated crew with optimal pre-flight training and coordination in effective communication, problem-solving, etc.). Therefore one of the focuses of the projects on the Team is to reduce psychosocial risks by identifying the psychological and behavioral features of individuals and small groups that result in optimal behavioral effectiveness under ground-based (analog) conditions comparable to space flight. This approach is taken in the Antarctic project directed by Dr. JoAnna Wood. Other projects directed by Dr. Joe Brady, Dr. Judith Orasanu, and Dr. James Carter seek to determine ways to prevent or resolve psychosocial miscommunication within teams and between space-bound teams and ground controllers.

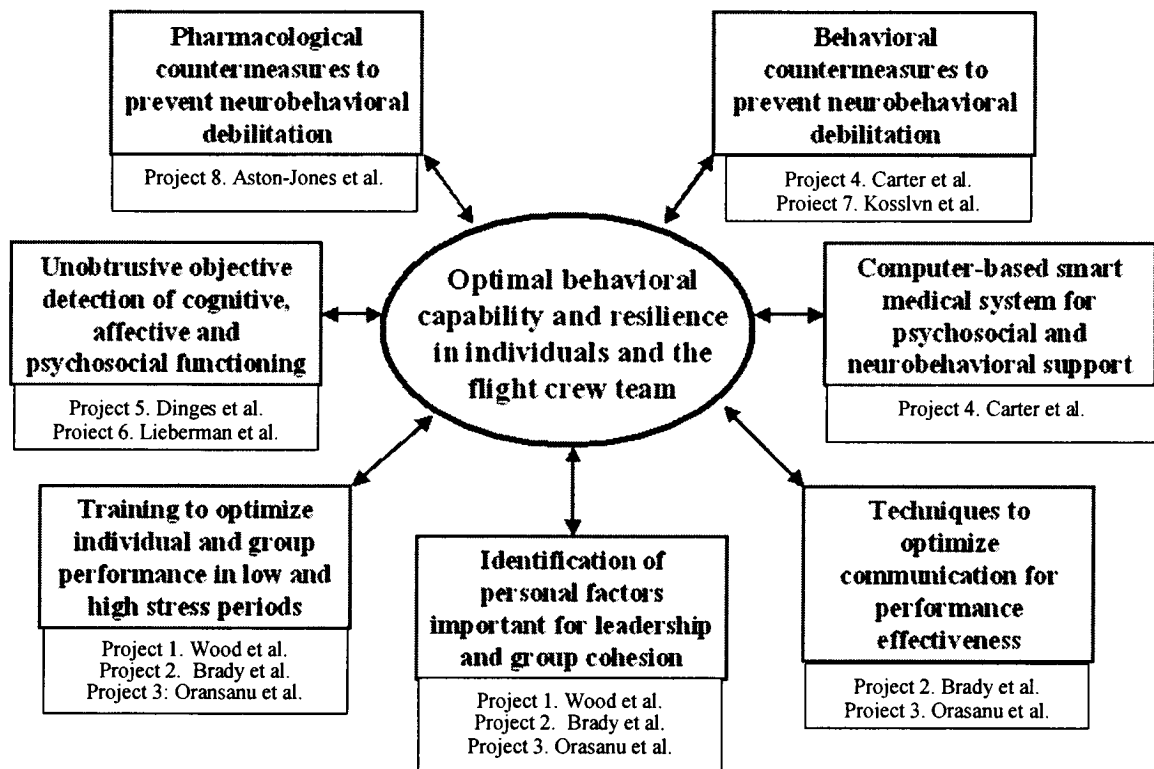
While prophylaxis against the development of neurobehavioral and psychosocial problems is ideal, there is no way to guarantee that preventative strategies alone will suffice. The Team therefore also has a strong focus on early detection and resolution of neurobehavioral problems and psychosocial conflicts. Especially critical is the need for reliable, objective measures of neurocognitive and emotional states and stress reactions. The projects directed by Dr. David Dinges, Dr. Phillip Lieberman, and Dr. Stephen Kosslyn deal directly with obtaining these measures. The emergence of either thought or mood disorders during space flight is likely to be a low-probability event, but if it occurs, it poses very serious risks not only for the individual's behavioral capability but also for the team's performance. Both neurobehavioral and psychosocial problems have occurred in long-duration space flight—with the latter common enough to have resulted in its rating as among the most serious risks (i.e., Type I) in the Critical Path Roadmap; consequently, there remains an acute need to establish reliable, objective, unobtrusive methods for confirmation of stable cognitive and emotional functioning during prolonged human space flight. Without such information available to an astronaut, it will be difficult to ensure that appropriate neurobehavioral countermeasures (e.g., behavioral and pharmacological) will be deployed in a manner that maintains behavioral health. There must be redundant ways to ensure that neurocognitive, neurological, and neuropsychiatric problems that develop on orbit (regardless of the cause) are quickly identified and treated before they result in a loss of high-level performance capacity in a crewmember.

The Team also has a major non risk-based goal of finding optimal ways for crews to use communication modalities and techniques to maintain effective group functioning and problem solving within a flight crew and between the crew and ground controllers, family, and management. Effective communication can help maintain team performance in the face of adversity, and it is one of the best preventative and operational countermeasures for ensuring strong group psychosocial cohesion and performance. Like the development of novel objective, unobtrusive methods and approaches to monitor stress reactions, cognitive state, mood and performance in individual crew members, the establishment of maximally effective communication techniques for all types of contingencies in space flight will have significant relevance to a host of Earth-based problems.

The figure below illustrates the Neurobehavioral and Psychosocial Factors Team major research themes and anticipated countermeasure types of the current projects, as well as the projects relevant to each theme. Together the projects address critical complementary components to the maintenance of behavioral and psychosocial health and capability during long duration missions. The eight ground-based projects making up the Neurobehavioral and Psychosocial Factors Team have been underway an average of 30 months.

IV. TEAM ACCOMPLISHMENTS

For the most part, the projects on the Neurobehavioral and Psychosocial Factors Team have completed data acquisition and are in various stages of variables extraction and data analyses. Projects have begun to produce findings at an accelerated rate. In the past year, for example, the Team was collectively responsible for five peer-review publications and another nine are under review; four chapters; and 27 scientific presentations. Progress on each project during the past year is described below.



Project 1. Wood, J. et al.: Individuals and Cultures in Social Isolation.

This project addresses questions in Risk 18 in the NASA Critical Path Roadmap: Human performance failure due to poor psychosocial adaptation. The goal of the project is to increase understanding of the effects of personality, culture, and group characteristics on both individual and group performance in an extreme environment (Antarctica) that parallels many of the conditions likely to occur in long-duration space missions.

Progress: Investigators have completed the second year of the planned two years of data collection on N=100 volunteers. The following measures have been collected on the sample: personality scales; performance review scores; weekly psychological questionnaires; demographic and biological samples (on a subsample of n=80). The latter have been sent to NIH for processing; 85% have been assayed. Analyses of seven neuropeptides will start as soon as processing is completed.

During the past year data from weekly psychological questionnaires have been combined with similar data from previous studies in order to examine group effects and individual differences on several key outcome measures. One important finding to emerge from these analyses was that women and men differ in the effects of poor team cohesion on individual motivation to work. The relationship between team cohesion and motivation to work was quite strong in women, but nearly non-existent in men. Team cohesion was also related to leadership effectiveness such that good leadership is associated with strong team social cohesion and high work motivation for both women and men, while poor leadership was associated with low team cohesion and low motivation to work among women, but not men. Despite these gender differences in motivation to work, both women and men completed the work that needed to get done.

Implications: The relationships among leadership, team cohesion, gender, and motivation to work, while not surprising, have clear operational relevance. First, when team cohesion is low it places a greater burden on women than on men. They have to put forth more effort to accomplish their work. This situation may take its toll on women in greater physical fatigue, as well as other stress responses. Second, leadership behaviors can be changed, either by selection or training, to improve team cohesion and improve life for everyone.

Project 2. Brady, J. et al.: Psychosocial Performance Factors in Space Dwelling Groups.

This project addresses questions in Risk 18 in the NASA Critical Path Roadmap: Human performance failure due to poor psychosocial adaptation. The goal of the project is to determine the effects of variations in the structure and function of communication channels within and between simulated space-dwelling and Earth-based groups. It addresses the effects on psychosocial performance effectiveness of (1) variations in the structure and function of communication channels; (2) variations in positive and negative characteristics of incentive systems; and (3) selection, training and experience. The research methodology involves development of a distributed interactive multi-person simulation in computer-generated environments as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The simulated task environment (STE) provides an automated means of setting the context for the analysis of performance in space-dwelling groups and monitoring the effects of varying experimental conditions on psychosocial interactions.

Progress: Synthetic work scenarios based upon complex cognitive behavioral tasks generated and maintained cooperative and productive psychosocial interactions between individually isolated and dispersed crew members actively engaged in communicating and problem solving over extended time intervals without benefit of one another's physical presence. Single communication mode constraint studies completed this past year showed that there was a high degree of interchangeability between available modalities. When constraints were imposed simultaneously on multiple, frequently utilized modes of communication (e.g. audio and text) however, marked decrements in overall crew performance effectiveness were observed.

The results of experiments manipulating incentives under interacting stressful conditions in the synthetic task environment indicate that positive incentive countermeasures may be most effective when enhanced performance levels (e.g. working more quickly) are necessary to offset the time loss occasioned by distressing environmental events. Results were obtained with a computerized version of the visual analogue scale (VAS), a quantifiable self-report measure of behavioral disposition (e.g. 'feelings') associated with psychosocial adaptation. It was found that positive incentive conditions reversed progressively worsening negative ratings of being lethargic and stressed, as well as improved ratings of feeling happy and stimulated.

Implications: These results indicate that cooperative and productive interactions can be maintained between individually isolated and distributed group members communicating and problem-solving effectively in a computer-generated synthetic task environment over extended time intervals without benefit of one another's physical presence. The positive incentive countermeasure studies indicate further that such interventions may be most effective under conditions that require enhanced performance levels to offset the decremental time loss effects of stressful task environments.

Project 3. Orasanu, J. et al: Distributed Team Decision Making in Exploration Missions.

This project addresses questions in Risk 18 in the NASA Critical Path Roadmap: Human performance failure due to poor psychosocial adaptation. The project has two research goals: (1) to understand the effects of task and interpersonal stressors on team performance in challenging distributed decision-making situations and to identify effective strategies for coping with these stressors, and (2) to develop non-invasive technologies to detect low levels of mental and emotional stress in individual team members to allow the introduction of countermeasures before team dynamics deteriorate to the point of threatening mission success.

Progress: A simulated task environment (STE) was developed to address the objectives. The STE scenario involves a dynamic computer-based search and rescue mission set in Antarctica. It provided an ideal environment for studying team interaction and problem solving because its underlying cognitive demands reflect those of many real-world team tasks. A four-person crew must develop plans, manage resources, and collaborate in order to find a lost party, accomplish its mission, and cope with unexpected tasks. While participants work on the task, their physiological responses and facial expressions are recorded and monitored for signs of mental and emotional stress.

A baseline study involving twelve teams each consisting of four U.S. males (at least second generation American) was conducted. Participants worked together over a period of four days in the laboratory (21 hours total). Day 1 consisted of training on the computer-based task and an introduction to the physiological monitoring devices. On days two, three and four, participants worked through six scenarios, one easy and one difficult scenario per day. Half of the teams were induced to work in a fully cooperative manner. The other half received instructions and feedback designed to induce conflict between team members.

Team interaction and communication were analyzed from transcripts of audio- and videotapes of team planning and mission execution. Our initial review of team communication found both effective teamwork and negative affect. Effective task performance was found to depend on team collaboration, including a coordinated search strategy, communication about mission-critical information, task assignment and prioritizing, and plan adaptation based on successful strategies. Positive team climate and cohesion also were associated with effective performance. Negative behaviors associated with team failure included players being withdrawn and passive, engaging in competitive behavior and deceit, and instigating conflict through negative feedback, criticism, and public embarrassment or humiliation of others. Physiological responses (heart rate, R-R interval, muscle tension, skin conductance level, respiration, and pulse transit time) were monitored while players engaged in the search and rescue tasks. The time-coded output were filtered and cleaned prior to analysis. Statistical analyses are still underway, but initial observations revealed physiological reactivity coordinated with task stressors.

Implications: The results of our initial work show that (1) collaborative team behaviors can overcome task stresses in a distributed computer-mediated problem solving environment; (2) that negative interpersonal interactions in fact interfere with successful performance on a team task; and (3) that physiological reactivity to task and team stressors can be detected, thereby serving as a trigger for introduction of countermeasures. The present study provides integrated physiological, self-report, behavioral, and performance measures from teams working together over time on a common task. Performance models developed from these studies will advance theories of stress and team performance. Positive stress-coping behaviors extracted from the findings will serve as a basis for training crews to manage challenging team tasks and interpersonal interactions. Findings will be applicable to crew performance in long-duration

space missions, as well as in other isolated confined environments such as Antarctica or submarines. The ultimate goal of this project is to adapt the tools and techniques for use by space crews as self-monitoring and management systems.

Project 4. Carter, J. et al.: Designing a Smart Medical System for Psychosocial Support.

This project addresses questions in Risk area 18 in the NASA Critical Path Roadmap (poor psychosocial adaptation) and Risk area 21 (neurobehavioral dysfunction). The goal of the project is to develop a prototypical computer-based “psychosocial support system” for use in pre-flight training and onboard as a countermeasure resource. The primary aims of the final countermeasure system are to minimize psychosocial and neurobehavioral problems through prevention and intervention. The system will provide psychosocial countermeasures through training in the prevention and management of conflicts that can arise in flight and between the astronauts and ground crews. The system will provide neurobehavioral countermeasures through the development of a self-treatment program for mild depression, as well as training in how to recognize depression in one’s self and in others. Self-administered standardized measures of psychosocial problems (e.g., depression, conflict, and anxiety) will be included in order to guide users to relevant portions of the system. The system is being designed to be used confidentially, with results reported only to the user.

Progress: In the past 12 months consultation interviews have been completed with experienced long-duration flyers to specifying the content and design of the conflict and depression sections and the design of the overall system on paper. The on-paper design and content specification of the system are nearly completed, and are ready to move into media production. Production will commence as soon as a letter of award is issued by the NSBRI to Beth-Israel Deaconess Medical Center, where the project is now based (the P.I. moved from Dartmouth University to BIDMC this past year).

As currently designed, the top-level interface is an exterior view of the ISS, with five modules “repurposed” for the system and labeled as: Self-Assessments, Interventions, Training Simulations, Resources, and My Information. Clicking on any of these modules takes the user into it, where problem-focused content can be accessed. (At present, the problems addressed are conflict and depression.) The system is “open,” in the sense that users can move to any part they wish at any time, providing maximum flexibility and control, while being guided by mentors who suggest pathways through it, based on users’ expressed needs. Zooming interface technology enables users to keep track spatially of where they are in the system. This infrastructure will enable the expansion of the system to include new programs in the future addressing other problems.

Regarding the modules on conflict and depression, both include training content on how to prevent, assess, and manage these problems. The content was developed in conjunction with our subject-matter experts in these areas (Dr. Greenhalgh for conflict and Dr. Hegel for depression). The introductory script written for conflict management includes parts for two actors as well as the mentor, an animation, and an interactive activity. Additionally, the interactive simulation has branching simulations that permit users to “interact” with videotaped actors portraying crewmembers. Users choose how to respond to the virtual crewmember, with whom they have a conflict, and their choices determine how the simulation develops. The simulation now includes over 180 scenes, with 11 possible conclusions and dozens of routes through the simulation. Users go through between 6 and 18 choice points in any given route.

In addition to simulations, activities, and didactics, the depression program will feature a self help section in which the system guides users through the steps of Problem-Solving Treatment, in order to help them behaviorally treat their own depression.

Implications: Consultation interviews conducted with experienced long-duration flyers in Years 1 and 2 helped us to (1) understand the psychosocial environment on long-duration missions; (2) identify best practices for managing depression and conflict; (3) identify simulations to develop that are realistic and relevant to long-duration flight; (4) suggest courses of action to take in simulations; (5) identify differences between Earth and space in the management of depression and conflict; (6) introduce the study to long-duration flyers so that we gain their trust and support; and (7) identify interviewees to be videotaped who can provide advice and encouragement to users of the system. The progress made in the specification of the system's overall architecture plus specification of content and design of sections on content and depression has enabled transition to the production of media.

Project 5. Dinges, D. et al.: Optical Computer Recognition of Behavioral Stress.

This project addresses questions in Risk 21 in the NASA Critical Path Roadmap: Human performance failure because of neurobehavioral dysfunction. The goal is to determine whether optical computer recognition algorithms based on changes in facial expressions can discriminate behavioral stress induced by low versus high workload performance demands, and what influence gender, age ethnicity, alexithymia and other personality dimensions have on algorithm discriminability. The computer-based recognition system uses automatic optical tracking of human subjects' subtle anatomical and motor changes in facial expressions during cognitive performance tasks. Video input to the system is provided from experiments on healthy adults exposed to laboratory simulations of varying degrees of workload-based behavioral stressors.

Progress: During the past year data acquisition was completed. Performance workload was used to induce low and high stress in N=60 healthy adults (mean age 30y). Stress reactions were tracked during both low and high workload conditions using self-report visual analog ratings and mood scores, salivary cortisol and heart rate. Subjects completed questionnaires addressing aspects of personality, mood, stress perception and coping strategies, as well an alexithymia scale. High-resolution digital videos of subjects' faces in the low and high workload conditions were made and blind codified, to permit optical computer algorithm training and testing.

Results indicate that subjective stress was experienced via high workload demands. Ratings of general stress, task difficulty, effort required, and frustration increased significantly following the completion of the high workload test bouts compared to the low workload test bouts. In addition, high workload demands induced a significant elevation in all negative mood scores. Analyses are underway calculating global performance scores for subjects during the high and low workload bouts, in order to evaluate the relationship between actual performance levels during the high workload test bouts to the extent increased levels of psychological distress can be detected using the optical computer algorithm. Alexithymia (lack of emotional expression) was found to be related to subjective distress levels and to total mood disturbance during high performance demands, but unrelated to ratings of task difficulty, effort required, or frustration.

Significant progress was also made refining the deformable facial masks and the optical computer algorithm, to increase the sophistication of the optical computer recognition system to detect facial changes during performance stress. The final phase of the project, involving the application of the deformable masks and optical computer algorithm to video images of the subjects while performing in the low versus high workload conditions, is still underway.

Significant advances in programming and enhancement of the capabilities of the computer recognition algorithm have been completed, to develop a technique for robust 3-dimensional (3D) tracking of facial expression. The new technique we developed allows the translation of 2D video footage to a form that can be used for tracking the 3D orientation and translation of the face, as well as parameters that describe the movement of eyebrows, mouth, etc. (we published a major paper on this approach). This provides a sensitive and unobtrusive mechanism for the measurement of changes in facial expression during head movement, and allows us to test whether computer recognition algorithm detection of facial changes can be used as an accurate indicator of exposure to the high or low workload stressors.

Implications: If optical computer recognition of behaviorally induced stress responses can be demonstrated to work, we will have an unobtrusive technique for monitoring stress during performance onboard long-duration space craft. Two additional obstacles will need to be overcome to make this feasible in space. The first concerns the edema of the face due to fluid shifts in microgravity, and the second concerns the role alexithymia. The former concern will be addressed experimentally in a future study, while the latter is being evaluated in the current study.

Project 6. Lieberman, P. et al.: Speech Monitoring, Cognitive and Personality Alterations.

This project addresses questions in Risk 21 in the NASA Critical Path Roadmap: Human performance failure because of neurobehavioral dysfunction. The overarching goal of the research is the development of a monitoring system that predicts cognitive dysfunctions associated with deep space travel conditions. Cosmic radiation associated with deep space travel is likely to affect subcortical structures in the brain in a way similar to oxygen deprivation, that is, by compromising the function of basal ganglia circuits involved in both motor behavior and cognition. Speech is affected by small disturbances to motor circuits. By studying climbers on Mount Everest, a stressful, life-threatening environment analogous to the situation astronauts will face in deep space, we aim to develop and validate techniques for monitoring effects of neurological impairment via minute yet measurable changes in speech production. Studies of patients with Parkinson's disease allow the assessment of more profound impairment.

Progress: Several significant results were obtained over the past year from analyses of data collected on expeditions to Everest in 2001 and 2002. Among them are the following. Analyses of changes with altitude both in voice onset time parameters (VOT mean separation and VOT minimal distance) and cognitive performance revealed that subjects whose VOT precision worsened as they ascended the mountain were significantly more likely also to exhibit cognitive decline than subjects whose VOT precision stayed the same or improved during ascent. The predictive value of the speech measures was assessed for accurately detecting cognitive impairment. If both speech and cognitive measures signaled that a climber's performance was affected by hypoxia, or if both indicated an absence of any such effect, this was considered a "hit." When speech measurements indicated that a climber who showed unimpaired cognitive performance was affected by hypoxia, this was counted as a "false alarm." Finally, if speech measures failed to signal hypoxia when cognitive tests showed hypoxia-related decline, this was a "miss." Two speech measures showed promising results. Increased vowel duration had a hit rate of 85%, a miss rate of 15%, and a false alarm rate of 0%; decreased VOT mean separation had a hit rate of 73%, a miss rate of 7%, and a false alarm rate of 20%. We envision a future speech monitoring system that would exploit these relationships with even greater accuracy. We have also found a significant correlation between vowel duration and cognitive decline.

In spring 2003, the most recent Everest expedition was begun. This expedition incorporated the Mini-Cog cognitive test battery developed in Project 7 (Dr. Kosslyn). The Mini-Cog test battery provided nine cognitive tests assessing functions such as vigilance, divided attention, filtering, spatial working memory, and verbal working memory. The tests were programmed onto handheld Palm Pilots carried by the climbers for self-administration. The Palm Pilots also served as speech recorders. This additional technology allowed testing and recording even when severe weather obstructed radio transmission to research assistants at base camp. These data are currently being analyzed to determine if our earlier findings were replicated, and whether other aspects of cognitive performance on the Mini-Cog tests were affected by altitude and hypoxia. Analyses so far indicate that the most significant degradation occurs in performance on working memory tasks, both spatial and verbal. Mental Rotation, a task that requires mentally manipulating a pictured shape to determine whether it is the same as another shape or its mirror image, is minimally affected. This finding supports the notion that different neural circuits are differentially compromised by hypoxia.

Implications: Our findings show that cognitive dysfunctions arising from impairment to dopaminergic basal ganglia can be remotely monitored and assessed by means of acoustic measures of speech that are unobtrusive. Our measures reflect basic motor control deficits rather than the content of a message. The procedures can be used in the diagnosis and treatment of neurodegenerative diseases such as Parkinson's and may prove useful in evaluating cognitive dysfunction arising from task-induced stress in astronauts.

Project 7. Kosslyn, S., et al.: Quick Assessment of Basic Cognitive Functions.

This project addresses questions in Risk 21 in the NASA Critical Path Roadmap: Human performance failure because of neurobehavioral dysfunction. The goal is to develop a set of brief performance tests on a handheld device that will be computerized versions of standard tasks from the psychological literature, which tap a range of basic cognitive abilities. The performance tests being developed were selected from among standard tasks that tap abilities necessary for performing complex operations (such as EVA or many other jobs that astronauts have to complete) or that have been shown to be impaired by the types of variables that astronauts might expect to encounter in spaceflight. Ground personnel can also use these tests, as a way to alert them to the possibility of human error in operational tasks. Mini-Cog tests were designed to be self-administered, and the handheld program provides immediate performance feedback.

Progress: The second year of the project (the period covered by this report) focused on iteratively testing and refining different versions of the test battery (aided in part by a collaboration with Project 6) as well as refining the MiniCog application itself. Tasks were scripted using an off-the-shelf HTML editor, and then converted to a Palm OS compatible format using an interface provided by Bay Area Software. The MiniCog program, which runs on the Palm OS, has been modified in the following ways: The scoring algorithms are being expanded to handle tasks other than those where a "correct" or "incorrect" response is designated individually for each trial; stimuli can be randomly accessed from a pool much larger than the number that will be used in any given administration of the test (to prevent memorization of specific stimuli or responses); and users can take a series of tests without having to log in each time, and without viewing their scores. The greater memory available on new Palm Pilot models allows the data to be stored by user instead of by test (which makes detailed analyses easier); the data can also be stored on memory cards. The scripting will be made more flexible, and we will also be able to batch process the conversion of html scripts to Palm format files and Palm data files to text. Additional modifications (planned for the final year) include possible porting to Pocket-PC and other portable devices, as well as enhanced security features.

The specific tests implemented assess perceptual and motor control, attention (vigilance, divided attention, and filtering), verbal and spatial working memory, cognitive set switching, and verbal and spatial problem solving. Test revision has focused on adjusting the stimulus difficulty so that users do not get frustrated or bored; determining the number of times the tests must be performed before the user reaches a stable baseline; developing color-independent tasks; comparing performance on these tests with standard tasks from the literature, and collecting norms. In addition the collaboration with Project 6 (Dr. Lieberman) has led to Mini-Cog data acquisition from subjects ascending Mt. Everest, for use in determine whether hypoxia affects performance on any of the tests (see Project 6). Preliminary results from this collaboration lend validity to the Mini-Cog measures. In the last year of funding we plan to produce a final version of the MiniCog software, with documentation, for other investigators to use; to complete the normative data process and final data analysis; to produce documentation for use of the tests, including information about pre-testing to reach "baseline performance;" and, in conjunction with collaborators, to continue testing our battery under various field conditions.

Implications: Other researchers will be able to use the Mini-Cog test battery to assess cognitive performance quickly and under a variety of laboratory and field conditions; they may also use the MiniCog platform to develop their own psychological experiments. Both our test battery and the MiniCog application have an advantage over standard task batteries and many typical psychological scripting programs in that the tests are brief and the method of administration is extremely compact, portable, and fairly inexpensive. This could make MiniCog practical in a wide range of settings, such as spaceflight, where there are questions of neurocognitive capability.

Project 8. Aston-Jones, G., et al.: Stress, Performance and Locus Coeruleus.

This project addresses questions in Risk 21 in the NASA Critical Path Roadmap: Human performance failure because of neurobehavioral dysfunction. The goal of the project is to analyze locus coeruleus activity during a continuous performance task, to determine the effects of acute and repeated stress on changes in locus coeruleus function and performance, and identify pharmacological countermeasures to mitigate stress effects on locus coeruleus activity and attentional function.

Progress: Our previous results showed that clonidine at a low dose (1 $\mu\text{g}/\text{kg}$) prevented performance decrements produced by restraint plus noise stress in a continuous performance-attention task in rodents. In the past year, we have found the following.

Repeated testing of the same animals revealed that individuals consistently exhibited similar stress deficits, indicating that stress effects on performance may be a trait of the individual animal.

Like results for noise-restraint stress, total sleep deprivation produced increased errors (false alarms) in task performance, and clonidine (1 $\mu\text{g}/\text{kg}$, ip) mitigated these deleterious effects of sleep-deprivation stress on performance.

The effects of sertraline (10mg/kg, ip) on performance deficits in sleep-deprived rats was also evaluated. Sertraline failed to significantly reverse the effects of sleep loss on performance, although a tendency to attenuate the performance deficit was found.

Implications: These results indicate that low-dose clonidine may be an effective treatment for stress-induced performance deficits in an animal model. Since high doses (above 1 $\mu\text{g}/\text{kg}$) produce sedation, these are not recommended. Sertraline may also be effective, but more studies with a wider range of doses are needed to further test this possibility. Clonidine limits activity of locus coeruleus neurons and release of norepinephrine, and sertraline has a similar effect (via the inhibitory effects of serotonin on locus coeruleus activity). Therefore, these results are consistent with the overall view that altered locus coeruleus activity may underlie stress-induced performance deficits, and provide a good target for developing appropriate countermeasures.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Research Team Annual Report November 3, 2003

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One signed original, singled-sided copy with no binding, suitable for reproduction plus one electronic copy in Microsoft WORD for PC .

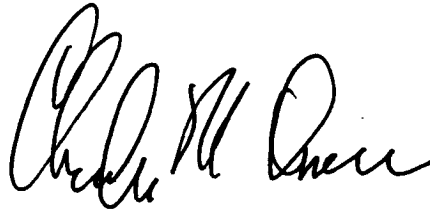
A handwritten signature in black ink, appearing to read "Paul Lampi". The signature is written in a cursive style with a large initial "P" and "L".

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I. ABSTRACT

The most overt change affecting an astronaut in space flight is the immediate response of the neurovestibular system to changes in gravity. NSBRI's neurovestibular adaptation research program is aimed at developing scientifically-based countermeasures against the vestibular problems associated with space flight. Problems typically arise first when astronauts transition from 1-G to 0-G, unfortunately at a time when their physical and cognitive performance is often critical for mission success and safety. Postflight problems have generally been more severe after 3-5 month Mir and ISS flights than on 1-2 week Shuttle missions, showing that some components of vestibular adaptation to 0-G take place over time scales of months, rather than weeks. Operationally significant vestibular problems are also anticipated when astronauts make the transition from 0-G to partial G, or from 0-G to an artificial gravity environment. NSBRI research is designed to develop countermeasures for a broad set of risks identified by the team and the NASA Critical Path project. These currently are (in priority order): 1. Vertigo on reentry and landing, 2. Acute space motion sickness, 3. Postlanding imbalance, instability, vertigo and orthostatic hypotension or failed landing. 4. Inflight spatial disorientation and frame of reference problems, 5. Chronic space motion symptoms .6. Artificial Gravity related disorientation, nausea, vomiting, and loss of coordination. 7. Peripheral and central vestibular function changes.

The seven projects in the current portfolio have made significant progress. 133 journal articles, book chapters, abstracts and manuscripts have issued so far, including 47 peer reviewed journal articles, and are listed by project in Appendix 1. Some of the results were published in a special NSBRI issue of the Journal of Vestibular Research which appeared this fall, and others will appear next year in a special issue devoted to the proceedings of the Sixth Symposium on the Role of the Vestibular Organs in Space Exploration, held in Portland, OR, last fall, and co-sponsored by the NSBRI team.

In the context of countermeasure development, project highlights of programmatic significance during 2003 include:

- 1) Relative to re-entry and landing vertigo, Dr. Oman's project has demonstrated a new virtual reality based technique for direct measurement of head movement contingent oscillopsia. Subjects adjust the amount of scene motion produced by active head movements until the scene appears perceptually stable (CRL 5). The method can potentially be adapted to measure oscillopsia due to OTTR or G excess illusions. Dr. Reschke's project has shown that during eccentric gaze holding, the time constant of centripetal drift is dependent on the gravitational position. (CRL 3).
- 2) Relative to acute space motion sickness, Dr. Shelhamer's team continued parabolic flight experiments investigating possible changes in ocular vertical alignment (skew) which might cause diplopia and contribute to space sickness. (CRL 2), and continued to refine his experiments on inducing context specific changes in saccade gain. Dr. Reschke began to evaluate the use of electronic shutter glasses as a means of reducing sensory conflict during motion sickness (CRL 4)

- 3) Relative to postlanding vertigo and postural instability: In recent years, Dr. Wall's laboratory has developed a wearable prototype multi-axis vibrotactile balance aid suitable for use in walking experiments. With NSBRI support Dr. Wall investigated the use of a vibrotactile balance aid to recover from anterior-posterior balance disturbances while standing. The portable balance disturber ("BALDER") developed by Dr. Wall is now in use at JSC in Dr. Bloomberg's laboratory. Dr. Bloomberg continued his studies of visual acuity during treadmill locomotion, in subjects with both normal vision and while wearing x 0.5 minifying lenses, and at distances of both 0.5 and 4 meters. They found that subjects are able to modify full-body segmental kinematics in order to reduce head perturbations. These locomotion and visual acuity assessment techniques will be evaluated by the NSBRI Integrated Team for use in the postflight Clinical Status Evaluation (CSE), with the goal of obtaining normative postflight locomotion data for use in assessment of emergency egress capability, fitness for return-to-duty, and efficacy of rehabilitation. (CRL7). Bloomberg's treadmill visual acuity test is also scheduled for an inflight evaluation (ULF-2, about 2005). Dr. Shelhamer investigated measurement of the gain of the oculomotor response to transient head motions (lateral heaves and fore-aft surges), which might form the basis of a tool for postflight assessment of otolith and cerebellar function.
- 4) Relative to inflight spatial disorientation and frame of reference problems: Drs. Oman's project developed new methods to quantify the direction of the perceived vertical using the "shape-from-shading" illusion, useful for assessment of the effects of polarity and frame cues provided by visual scenes (useful for design of spacecraft interiors and workspaces). They demonstrated the feasibility of a virtual reality based 3D spatial memory training countermeasure, comparing the effectiveness of randomized vs. blocked presentations (CRL 6). They also showed that rat head direction cells exhibit a distinct "disorientation" response state, corresponding to Type II/III spatial disorientation in humans.
- 5) Relative to chronic space motion sickness, Dr. Dornhofer's project demonstrated that the midlatency auditory evoked P50 potential (a measure of the ability to filter out familiar sensory stimuli) changes during motion sickness onset, that the benzodiazepine Lorazepam (1 mg, p.o.) was ineffective at preventing Coriolis induced motion sickness, as compared to promethazine.
- 6) Relative to the development of short radius Artificial Gravity as a multisystem countermeasure: Drs. Young's project has shown that 75% of normal subjects can tolerate single axis head movements at 23 RPM, and that incrementing the RPM while adapting reduces symptoms. They demonstrated that motor adaptations are rapid, that VOR adaptation to Coriolis stimulation requires a visual scene, that the angular VOR time constant shortens, and that adaptation transfers to some degree across two different directions of centrifuge rotation, important since astronauts ideally should remain oriented and asymptomatic regardless of the orientation of their head with respect to the direction of the centrifuge velocity vector.

- 7) Relative to new methods for assessment of chronic vestibular function changes, Dr. Dornhofer's project has evaluated measurement of 3D eye movements and deviation of the subjective visual vertical during off axis angular rotation testing. They showed that the SVV in patients with unilateral lesions deviates ipsilaterally (CRL 5).

II. INTRODUCTION

The most overt change affecting an astronaut in space flight is the immediate response of the neurovestibular system to changes in gravity. NSBRI's neurovestibular adaptation research program supports research aimed at developing scientifically-based countermeasures against the vestibular problems associated with space flight: spatial disorientation, space motion sickness, oculomotor deficits, postflight postural instability and gait ataxia. Problems typically arise first when astronauts transition from 1-G to 0-G, unfortunately at a time when their physical and cognitive performance is often critical for mission success and safety. Postflight problems have generally been more severe after 3-5 month Mir and 4-6 month ISS increments than on 1-2 week Shuttle missions, showing that some components of vestibular adaptation to 0-G take place over time scales of months, rather than weeks. Operationally significant vestibular problems are also anticipated when astronauts make the transition from 0-G to partial G, or from 0-G to an artificial gravity environment. During the Shuttle/Spacelab era (1980s and 90s), many of NASA's major ground and flight neurovestibular experiments addressed basic issues related to the effects of 0-G vestibular reflexes. NSBRI research is designed to develop countermeasures for a broader set of risks identified by the NASA Critical Path project. In 1997, five neurovestibular risk areas were identified (criticalpath.jsc.nasa.gov/main.asp). Since that time, additional information has become available from long duration Shuttle, Mir and ISS flights. Because of NASA's shift in emphasis from exploration (e.g. Mars) missions to long duration (1 year) ISS long duration flights, the NSBRI neurovestibular team reexamined its critical path risks. In 2002 we regrouped the risks into seven areas which also define the spaceflight related long term goals of the current program. In priority order, these are:

1. Vertigo on reentry and landing, triggered by sudden vehicle accelerations or head movements in a now-unfamiliar gravitational environment, can cause involuntary eye movements (nystagmus), difficulty reading instruments, oscillopsia (illusory movement of the visual scene), and orientation illusions. Together, these can cause misperception of the attitude, velocity, and acceleration of the vehicle. In critical situations, such as during landing they can lead to involuntary control movements and control errors, resulting in faster and harder landings and potentially in the loss of the vehicle and crew.
2. Acute space motion sickness on insertion into microgravity can produce nausea, vomiting, loss of concentration and inability to follow procedures. The sickness, which can sometimes last for several days, could cause catastrophic failure of EVA suit life support systems and render space suits un reusable, were sickness to occur during EVA. Consideration of this has caused deferment of non-emergency EVAs and Shuttle rendezvous and docking to the fourth day of flight, which has an important impact on Shuttle procedures and crew productivity.
3. Postlanding imbalance, instability, vertigo and orthostatic hypotension have made some crewmembers unable to stand up or walk unassisted after medium and long-duration

flights. Associated with this, there is decreased tone in postural muscles, impaired locomotor coordination, instability of vision, difficulty turning corners or negotiating stairs. Any or all of these compromises the ability of crewmembers to egress from the Shuttle rapidly. Potentially, this could lead to injury or death of crewmembers in the event of an emergency or failed landing.

4. Inflight spatial disorientation and frame of reference problems, triggered by 3D body movements as well as inversion and visual reorientation illusions, causing reaching errors and spatial memory problems, difficulty locating emergency egress routes, EVA height vertigo, and operational difficulties during docking and remote manipulation of payloads that could cause dangerous collisions.
5. Chronic space motion symptoms resulting in decreased crew work capacity. Symptoms include fatigue, “space stupids”, decreased vigilance, loss of motivation, irritability, gastrointestinal stasis, anorexia, dehydration, weight loss, side effects of anti-motion sickness drugs and changes in sleep-wake cycle.
6. Artificial Gravity related disorientation, nausea, vomiting, and loss of coordination. Symptoms occur in short and medium radius artificial gravity environments due to Coriolis effects on the vestibular semicircular canals, and biomechanical Coriolis forces which disturb normal limb movements. Symptoms necessitate movement restrictions which will compromise crew productivity.
7. Peripheral and central vestibular function changes due to exposure to microgravity, that may contribute to orthostatic intolerance on landing. It is also conceivable that changes in otolith or hair cell function occur after very long duration exposure to weightlessness, or exposure to radiation and environmental ototoxins (e.g. CO) that could cause permanent impairment of balance function. If so, these changes could cause loss of crew productivity when landing on a distant planet or crew injury or death during emergency egress.

III. TEAM STRUCTURE AND DESIGN

Design:

The ultimate goal of NSBRI's neurovestibular research program is to develop countermeasures that ultimately will allow crewmembers to: avoid disorientation, meet the physical requirements of emergencies, treat motion sickness without side effects, and safely control vehicles and systems.

Risk #1 (Vertigo on Reentry and Landing) is believed to represent a serious (“Red/One”) risk on long duration missions. Though Shuttle pilots are aware of the problem, and voluntarily limit head movements, vehicle accelerations cannot be avoided. Vertigo and nystagmus cause well known difficulties reading flight instruments. Reentry vertigo has been recognized since the earliest days of the Shuttle program, but its operational

significance has probably been masked by the traditional “can do” attitude of military-trained pilots who believe they can concentrate on their instruments and “fly through” episodes of vertigo. Flight surgeons report that vestibular disturbances are more severe after long flights. Although the landing vertigo problem has been manageable on 1-2 week flights, it is likely to become a significant problem if shuttle mission duration is extended to 3-4 weeks. McClusky, Clark, Stepaniak 2001 (NASA JSC SD2) found shuttle landing flight technical error (height over threshold, and distance, vertical velocity, and airspeed speed errors at touchdown on 9 missions) correlated with intensity of postflight neurologic symptoms. Vertigo on short final, flare, or touchdown could cause loss of vehicle control. Vestibular and related somatosensory factors may have contributed to pilot induced oscillations on some Shuttle landings. Additional quantitative data on head movements, vehicle accelerations, and flight technical error are needed. The Shuttle does not have full autoland capability at all likely landing sites. Countermeasures to pre-adapt crewmembers or display/flight control changes and training procedures which reduce disorientation and flight technical error will be required. Providing Shuttle autoland capability will not completely resolve the problem, since pilots must still have sufficient visual acuity to monitor displays used in landing.

Risk #2 (Acute space motion sickness) also represents a Class I risk during EVA, since the Shuttle space suit (“EMU”) has no containment bag. In 1980s, Hamilton Standard noted vomitus in the LiOH canister creates exothermic reaction, and shuts down EMU primary vent loop. Frozen vomitus in secondary vent nozzle could shut down the secondary vent loop, leaving only a few minutes of residual in suit O₂ remaining. Vomitus is biologically active, so if there is an episode, the suit cannot be reused unless completely refurbished on the ground. Vomitus volume could be somewhat reduced by eating/drinking less frequently, but this is often inappropriate. Modifying the suit to include a vomitus containment receptacle was considered in the 1980s but was deemed too expensive and impractical. Risk is serious if emergency EVA is required. Risk exposure currently is currently reduced by prohibition of non-emergency EVAs before flight day 3. One in-suit vomiting episode has occurred, but before actual EVA began. Acute vomiting episodes – even during IVA – are momentarily disabling. Drug or behavioral countermeasures which reliably and quickly reduce probability of vomiting are needed. Feasibility of EMU modifications to reduce susceptibility or provide containment need to be reinvestigated. Opening the early-mission window for EVA by 1-2 days will add useful flexibility in mission planning, and improve overall STS-ISS productivity.

Risk #3 (Postlanding imbalance, instability, vertigo) remain a concern for all Shuttle crewmembers in the event an emergency requires rapid egress from the vehicle. Although recent cardiovascular and neuromuscular countermeasures have been successful on ISS, neurovestibular balance problems remain a problem for some individuals. Many crew tested cannot run 1000 ft on a treadmill. Countermeasures are needed to pre-adapt returning crewmembers, to mitigate the risk of injury resulting from an accidental fall. It is also important to understand whether there is a vestibular contribution to postflight orthostatic hypotension.

Risk #4 (Inflight spatial disorientation and frame of reference problems) are more significant inside space stations (Mir, ISS) than on Shuttle, due to the complex 3D interior architecture, which provides multiple visual frames of reference, and causes visual reorientation illusions. Mental rotation and frame of reference problems have been noted in debriefs of some crewmembers doing ISS robotic ops. Such problems complicated the Mir crew's response to the collision with the Progress spacecraft in 1997. Shuttle crewmembers visiting Mir easily became lost. Mir and ISS crewmembers occasionally report height vertigo when the Earth is in their lower visual field, and for some the experience has been momentarily disabling. The lack of visual references cues during the dark half of each orbit has caused disorientation and concern among some ISS EVA crew. Potential countermeasures include preflight visual orientation training – perhaps using appropriate virtual reality techniques or ground simulators – and improved physiologically based human factors standards for spacecraft architecture and escape path signage.

Risk #5 Chronic space motion sickness symptoms affect 75% of crewmembers to some degree during the first 3-5 days in space, and impair the average physical and mental efficiency of crewmembers, and cause profound somnolence, nausea related inability to follow procedures, and loss of initiative. The impact on operational capability of the crewmember equals or exceeds the somnolence produced by other aberrant circadian cues associated with spaceflight. Though acute space sickness problems are generally confined to the first week, several cases lasting weeks have been described by Russian colleagues, and there is reason to believe chronic low grade symptoms (“sopite syndrome”) may persist in some crewmembers for weeks. Existing drugs were developed to prevent and treat acute space motion sickness, and have significant side effects. They may not be the best agents for treating chronic space motion sickness symptoms. Countermeasures include both techniques which accelerate adaptation to weightlessness, and improved anti-motion sickness drugs and other therapies which can be used to block or treat symptoms and signs without unacceptable cognitive or circadian side effects.

Risk #6 Artificial Gravity related disorientation, nausea, vomiting and loss of coordination. Artificial gravity (AG) remains a potentially important multi-system countermeasure for neuromuscular, bone, cardiovascular and neurovestibular dysfunction in 0-G. Large radius AG spacecraft systems are likely at least a decade away, but short radius (2-3 m) systems could be developed now which fit inside Shuttle or an ISS module. As a neurovestibular countermeasure, AG is a double-edged sword: it probably can be used to pre-adapt crewmembers for return to planetary gravity, but if crewmembers move their heads out of the plane of rotation, the resulting vestibular Coriolis stimulus potentially produces complex disorientation and motion sickness. In a rotating artificial gravity environment, with the body's principal oriented perpendicular to the axis of rotation, the direction and magnitude of the vestibular Coriolis effects depend on which way the crewmember happens to be facing. The extent to which a person can adapt in a context specific way to this kind of stimulus is unclear, and requires further research. Establishing the values of AG system radius and RPM, and the duration/repetition rate

of AG sessions which are effective for neuromuscular, bone, cardiovascular and neurovestibular therapies remain a NSBRI wide priority.

Risk #7 Peripheral and central vestibular changes due to prolonged 0-G, radiation, or environmental toxins. There is no conclusive evidence that prolonged (months to years) exposure to 0-G produces irreversible vestibular changes, but only half a dozen individuals have yet flown beyond 6-8 months. Anatomical changes have been seen in vestibular sensory epithelia in animals on flights of several weeks and longer, but the functional significance of these changes is unclear. The effects of radiation exposure on the vestibular end organs and central vestibular system (e.g. brain stem, cerebellum, thalamus, hippocampus) has not been established. The effects of gravity on the formation of otolith crystals is not well understood. Loose otoconia will presumably float benignly in 0-G, but returning crewmembers could be more susceptible to benign paroxysmal positional vertigo due to canalithiasis. The lack of validated, sensitive instrumentation and methods for early detection of impairment of vestibular reflexes, particularly those associated with response to gravity and linear acceleration is a continuing problem.

In 1999, the NSBRI neurovestibular team held a workshop in Houston to solicit the advice of a panel of outside experts. This group mapped the neurovestibular risks of spaceflight into eight interrelated thematic research areas, and defined a set of critical questions associated with each. These are important, as they formed the basis for the solicitation of the current research program:

1. Sensory-Motor Adaptation

- Can an individual's ability to adapt to multiple gravitational environments be enhanced so astronauts can rapidly transition between 1-G and 0-G, 0-G and partial G, or 0-G and artificial G with minimal performance impairment or motion sickness? What are the sensory-motor responses that must change in a functionally adaptive manner during prolonged space flight? Does such adaptation take place? How can it be reliably measured?
- Can preflight or inflight training accelerate adaptation? Can these adaptive responses be trained to be context-specific? What context cues are effective? Must they be associated with active movement? How long does context-specific pre-adaptation last? Does adaptation of eye movements transfer to e.g. arm movement?
- What is the evidence for and the physiological bases of oscillopsia, disorientation, ataxia, impaired gaze holding, and reduced dynamic visual acuity reported by crewmembers, particularly while making head movements during re-entry and immediately postflight?
- Can long-term exposure to space flight impair sensorimotor plasticity?
- What is the mechanism responsible for postflight sensory flashbacks occasionally reported by some crewmembers?
- How do countermeasures (e.g., artificial gravity, inflight exercise or preflight training) affect adaptation rates and levels? How do rates and levels associated with

physiological (sensorimotor, autonomic, emetic) adaptation to microgravity and 3/8 G on Mars correlate with operational performance changes ?

- What are the appropriate space flight analog environments that can be used as test beds for evaluating neurological adaptation, adverse operational implications, countermeasures and impacts of adaptation on other anatomical and physiological systems?

2. Artificial Gravity

- What are the effects of AG on human eye, head and limb movements ? What are the pros and cons of artificial gravity (AG) as a countermeasure against the effects of 0-G on neurovestibular function ? What are the advantages and disadvantages of large radius continuous AG vs. short radius intermittent AG, and how are these influenced by mission duration and post-landing environment (Mars vs. Earth)?
- Can humans successfully adapt to working perpendicular to the angular velocity vector?
- How can transitions between AG levels be eased?
- What is the maximum tolerable rotation rate for a given G level? What is the best habituation schedule?

3. Visual (Multisensory) Orientation, Spatial Memory, and Navigation

- How do visual and nonvisual cues interact to influence human orientation perception and motor behavior?
- How do visual, vestibular and haptic cues and biases contribute to inversion illusions, visual reorientation illusions, extravehicular-activity acrophobia, disorientation and poor 3-D spatial memory in 0-G?
- What is the neural basis of inversion illusions, visual reorientation illusions, EVA acrophobia, disorientation and 3-D spatial memory problems in 0-G? Does neural coding of place and direction three dimensional, or is it principally two dimensional due to our terrestrial evolutionary heritage ? Does the coding change after adaptation to 0-G?
- Does 1-G training in simulated environments (e.g. using virtual reality or neutral buoyancy techniques) reduce disorientation, and improve 3-D spatial memory and performance in orientation and navigation tasks such as emergency escape ? Can the architecture and layout of spacecraft interiors be improved to minimize disorientation ?
- How can 0-G immersive teleoperation displays be designed to reduce disorientation and/or motion sickness?

4. Vestibular/Autonomic/Emetic Physiology and Countermeasures

- What is the physiological basis for the “sensory conflict” theory for motion sickness ? What is the locus and function of the putative “conflict” signal ? What is the neural or chemical linkage between balance and emetic centers ? What mechanisms establish the threshold for nausea and emesis ? What neurotransmitter and receptor systems are involved ? Is the physiology of space motion sickness fundamentally different from other forms of motion sickness ?
- How do anti-motion sickness drugs affect sensory-motor adaptation and eye movements ?

- Can more effective anti-motion sickness drugs be developed which target emetic centers or the vestibular-emetic linkage ? Drugs must be effective, easily and safely used over days to weeks with minimal side effects and must not impair neurovestibular adaptation.
- Can improved anti-motion delivery systems and dose and side effect monitoring systems be developed? What are the best ground-based techniques for evaluating 0-G pharmacokinetics and for assessing the effectiveness and side effects of drug countermeasures ?
- How does chronic space motion sickness (including sopite syndrome) affect mood, initiative and interpersonal relationships?
- Does the neurovestibular response to weightlessness impair postlanding cardiovascular regulation and contribute to orthostatic intolerance ? How is it mediated ? What is the effective frequency range of compensation ? Can an effective countermeasure (e.g., AG) be developed to exploit this knowledge?

5. Postflight Locomotion and Gaze Assessment

- What causes the profound impairments of posture, gaze and locomotion stability in many returning astronauts (and in vestibular patients), and how can these be quantified?
- What causes the large differences in level of impairment observed among different crewmembers ?
- How do these differences correlate with physiological and operational performance changes?
- How are the multiple, mutually dependent sensorimotor systems responsible for locomotion altered by exposure to space flight? For example, what is the role of the vestibulo-ocular, vestibulo-collic and vestibulo-spinal reflexes in 3-D control of locomotion and gaze while walking, turning or ascending stairs?
- How are target acquisition, smooth pursuit and saccadic mechanisms programmed during locomotion? How do oculomotor and gait control systems interact during locomotion and head turning? How is this interplay affected by space flight?
- What roles do visual cues play in postflight locomotor control?
- In an altered sensory environment, does motor control require increased cognitive resources?
- Does this multi-tasking impair performance? Can a dual-task paradigm be used to monitor adaptation?
- What is the linkage between space flight-induced changes in sensory-motor control and astronaut functional performance?
- What measures represent composite and global indicators of locomotor and/or gaze dysfunction after space flight? What measures are the most efficient and sensitive indicators of changes in locomotion and/or gaze? What is their correlation with functional performance after space flight.

6. Neurovestibular Rehabilitation

- What are the relative contributions of neurovestibular adaptation, neuromuscular deconditioning and orthostatic intolerance to postflight neuromuscular coordination, ataxia and locomotion difficulties?

- Why do certain astronauts recover balance and locomotion function more rapidly than others postflight ?
- What is the effect of cardiovascular, muscle and skeletal rehabilitation therapies on neurovestibular recovery, and the converse ?
- Can preflight or inflight training, balance exercises, sensory aids, prostheses and assessment techniques improve postlanding postural and locomotor control and functional task performance ?
- How should somatosensory information be used to accelerate neurovestibular readaptation?
- Can crewmembers “learn how to learn” by adapting to surrogate sensory-motor rearrangements ?
- How does attention to a new sensory-motor task affect performance of a secondary task?

7. Effects of Stress, Isolation, Immobilization and Diet on Vestibular Function

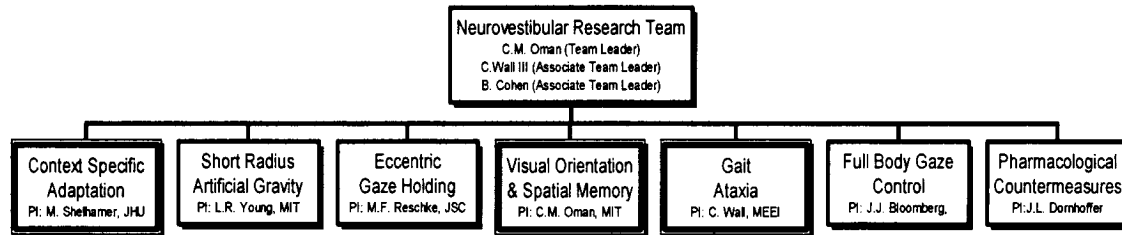
- What are the effect of psychological stress, isolation, immobilization, and diet on vestibular function ? How can they be distinguished from the effects of weightlessness and “normal” physiological variability?
- If there are important effects, what countermeasures can be developed?

8. Potential Mechanisms For and Diagnosis of Irreversible Neurovestibular Changes

- How might very long duration exposure to 0-G or partial G, radiation or environmental toxins such as carbon monoxide or ethylene glycol cause irreversible (pathophysiological) changes in central or peripheral vestibular function or development, or cause acceleration of the normal aging process? What is the likelihood of this ? Would some individuals be more susceptible than others? What is the potential time course? How could such changes be reliably detected at an early stage? What is the best way to non-invasively assess the function of the human otolith end-organs ? How does serum calcium homeostasis impact otoconial turnover?

Current Structure:

NSBRI's neurovestibular research program is led by Dr. Charles Oman (MIT) assisted by Drs. Bernard Cohen (Mt. Sinai School of Medicine) and Conrad Wall (Harvard Medical School/Mass Eye and Ear Infirmary). The current research portfolio of seven projects was selected based on a February, 2000 solicitation (NSBRI 00-01) and independent peer review. Six of the seven are three year projects. Three of the projects (double boxed in the figure below) were initiated in 1997, and competitively renewed in 2000. Completion dates of the projects range from December, 2003 to September 2004 due to NSBRI funding problems encountered in FY2002 and 2003, and in some cases delayed starts.



The investigators, their institutions, the critical path risks addressed, thematic areas, experimental model, specific aims, countermeasure types and countermeasure development strategy of each of the seven projects are summarized below:

Context-Specificity and Other Approaches to Neurovestibular Adaptation.

PI: Mark J. Shelhamer,

CoIs: Minor, Zee, Angelaki, Zhou, Wu.

Institutions: Johns Hopkins U. School of Medicine, Washington U., U. Mississippi Med Ctr.

Critical path risks addressed: 1. Vertigo on reentry and landing., 3. Postlanding imbalance and vertigo.

Thematic area: Sensory Motor Adaptation

Experimental models: Human and animal (primate)

Countermeasure Types: assessment, prediction, training.

Specific Aims:

- Is torsional eye position a context cue for saccade adaptation ?
- Does a rest interval between stimuli promote adaptive consolidation ?
- Can cyclovergence adaptation provide a countermeasure to ocular torsion changes in parabolic flight ?
- How do pursuit and LVOR deficits correlate in cerebellar lesioned monkeys ?
- How do pursuit and LVOR adaptation transfer across frequencies in humans and monkeys
- Can LVOR adaptation be trained with pursuit stimuli, and how do cerebellar lesions influence adaptation.
- Does head tilt adaptation of saccades and VOR transfer to arm movements in monkeys ?
- What is the best way to induce context specific LVOR adaptation in humans ?
- Does the naso-occipital LVOR also show context specific adaptation ?

Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation

PI: Laurence R. Young.

CoIs: Hecht, Oman, Mast, DiZio, Lackner, Paloski, B. Cohen,

Dai, M. Cohen, Welch, Stone.

Institutions: MIT, Brandeis, NASA-JSC, Mt. Sinai Hospital, NASA-Ames.

Critical Path Risks: 6. Artificial Gravity, 2. Acute space motion sickness, 5. Chronic space motion sickness

Thematic areas: Artificial G, Drug countermeasures

Experimental model: Human

Countermeasure Types: assessment, training, environmental manipulation, drugs.

Specific Aims: Using short and medium radius centrifuges and rotating chairs, to determine:

- How context cues influence VOR, perception and motion sickness adaptation.
- What is the role of sensory-motor (non-vestibular) adaptation to AG ?
- What types of sensory conflict drive adaptation ?
- What are the optimal duty cycles and inter-session intervals ?
- Does body orientation re gravity provide a context cue ?
- In what way does adaptation generalize to different rotating environments?
- How does intermittent training influence the accuracy of head movements ?
- How does promethazine affect adaptation and eye movements in humans and monkeys ?

Modification of Eccentric Gaze-Holding.

PI: Millard F. Reschke

CoIs: Paloski, Kornilova, Wood, Leigh

Institutions: NASA-JSC, IBMP/Moscow, BCM, University Hospitals of Cleveland

Critical Path Risk: 1 Vertigo on reentry and landing, 3, postlanding vertigo, 7. peripheral or central vestibular changes.

Thematic areas: Sensory-motor adaptation, Irreversible changes).

Experimental model: Human

Countermeasure types: assessment, prediction, training.

Specific Aims:

- Effect of tilt and proprioception on centripetal drift time constant
- How rebound nystagmus provides adaptive compensation.
- How centrifugation influences gaze holding.
- Why adaptation fails in cerebellar patients.
- Whether gaze-holding is impaired immediately following spaceflight.

Visual Orientation and Spatial Memory.

PI: Charles M. Oman.

CoIs: Howard, Shebilske, Taube, Hecht, Harris, Jenkin, Liu, Stuerzlinger. Institutions MIT, York University, Dartmouth Medical School, Wright State University.

Critical path risk: 4. Inflight spatial disorientation and frame of reference problems, 2. Acute space motion sickness etiology.

Thematic area: Orientation and Spatial Memory.

Experimental models: Human and animal (rat)

Countermeasure types: assessment, prediction, training, environmental manipulation. .

Specific aims:

- Human visual orientation. Effects of visual frame, polarity, brightness, motion, and gravireceptor cues on the subjective vertical, eye movements, and limb movements.

- Three dimensional spatial memory and spatial frameworks. Generic and environment specific preflight and onboard virtual reality training methods, interior architectural standards, and escape path countermeasure design and evaluation.
- Neural coding of spatial orientation. How do visual, vestibular, gravireceptive, proprioceptive, and motor pathways drive limbic head direction cells in the rat, as a model for visual reorientation illusions in astronauts.

Advanced Techniques to Assess and Counter Gait Ataxia

PI: Conrad Wall III

Co-Is: Bloomberg, Oddson, Raphan, Solomon.

Institutions: Mass Eye and Ear Infirmary, NASA-JSC, Boston University, Mt. Sinai Hospital, U. Penn.

Critical Path Risks: 3. Postlanding imbalance, instability, vertigo.

Thematic area: Locomotion and gaze.

Experimental model: Human

Countermeasure types: assessment, prediction, training, prosthesis.

Specific Aims:

- Quantify body, head, & eye coordination during perturbed straight walking. And also:
- during straight and circular walking on a circular treadmill.
- while ascending/descending stairs.
- while wearing a tactile prosthetic countermeasure.
- assess effect of dynamic balance exercises.

Understanding Full-Body Gaze Control During Locomotion

PI: Jacob J. Bloomberg, Jacob

Co-I: H. Cohen.

Institutions: NASA-JSC, Baylor College of Medicine

Critical Path Risks: 3. Postlanding imbalance, instability, vertigo, and hypertension.

Thematic area: Locomotion.

Experimental model: Human

Countermeasure types: assessment, prediction, training.

Countermeasure Readiness Level: 5

Specific Aims: How are eye, head, trunk, and lower limb movements coordinated.

Specifically:

- How do eye, head, trunk, and legs absorb heel strike while treadmill walking? How do subjects adapt to magnifying and minifying lenses ?
- To reduced degrees of freedom, for example wearing a neck brace ?
- To wearing knee braces ?

Pharmacological Countermeasures for Space Motion Sickness

PI: John L. Dornhoffer

CoIs: Garcia-Rill, Paule, Van De Heyning.

Institutions: U. Arkansas for Medical Sciences, National Center for Toxicological Res., U. Hospital, Antwerp.

Critical Path Risks: 2. Acute space motion sickness, 5 Chronic space motion sickness.

Thematic area: Autonomic/drug

Experimental model: Human

Countermeasure types: assessment, prediction, pharmacological.

Specific Aims: (2 year project, ending July 2003)

- What are the effects of lorazepam, meclizine, promethazine, and scopolamine on coriolis induced motion sickness symptoms ?
- How do these drugs affect reticular sensory gating (P50 double click auditory evoked potential), time perception, short term memory, and learning ?

Each of the current projects resembles a small NIH Program Project Grant in that (Boomberg's project excepted) all involve multiple experiments conducted concurrently at several institutions, and significant collaborations between investigators. In addition, there are significant inter-project collaborations and coordinations. For example, Drs. Wall, Oddson and Bloomberg are coordinating their locomotion research, and developing a portable locomotion testing platform. Dr. Minor (Shelhamer project) assisted Dr. Taube in developing a semicircular canal blocked animal preparation. Drs. Shelhamer, Solomon, and B. Cohen and are working with JSC clinical colleagues on development of a postflight neurological assessment battery.

NEUROVESTIBULAR ADAPTATION RESEARCH PROGRAM

Project Research Activities

<i>PI/Project</i>	Risk(s) Addressed	Countermeasure Target	Experimental System	Focused Mechanistic Research	Preliminary Countermeasure Development Research	Mature Countermeasure Development Research
BLOOMBERG/ Understanding Full-body Gaze Control During Locomotion	<ul style="list-style-type: none"> Postlanding imbalance, vertigo, visual instability. 	<ul style="list-style-type: none"> Monitoring and Diagnosis (Prediction) Training 	Human, treadmill walking	Understand how body segments absorb heel strike, and adapt to VOR gain changes.	Determine how visual acuity changes during locomotion	Assess postflight locomotion and gaze performance and fitness for return to activities of daily living.
DORNHOFFER/ Pharmacological Countermeasures for Space Motion Sickness	<ul style="list-style-type: none"> Acute space motion sickness Chronic space motion sickness 	<ul style="list-style-type: none"> Pharmacological agents Diagnosis (prediction) 	Human, off axis rotating chair	Assess drug effectiveness and side effects on P50 AEP, memory, learning	Assess otolith responses measuring torsional eye movements in off axis rotating chair.	Recommend appropriate drugs and side effect assessment techniques.
OMAN/ Visual Orientation and Spatial Memory and Countermeasures	<ul style="list-style-type: none"> Inflight spatial disorientation & reference frame problems Acute space motion sickness Chronic space motion sickness 	<ul style="list-style-type: none"> Monitoring and diagnosis (assessment & prediction) Training Environmental manipulation 	<ul style="list-style-type: none"> Human, physical and virtual environments Animal (Long Evans rat) in 1-G and parabolic flight 	<ul style="list-style-type: none"> Assess role of frame & polarity cues in human visual orientation Determine 3D head direction cell coding in 1-G and 0-G. 	Develop 0-G 3D spatial memory and navigation training techniques.	Implement preflight and inflight orientation training techniques, path marking techniques, path interior layouts.

<p>RESCHKE/ Modification of Eccentric Gaze Holding</p>	<ul style="list-style-type: none"> • Re-entry & landing vertigo • Postlanding vertigo & visual instability • Peripheral & central vestibular changes 	<ul style="list-style-type: none"> • Monitoring and diagnosis (assessment & prediction) • Training 	<p>Human, normals and cerebellar patients, tilted and on centrifuge.</p>	<p>Determine how body tilt and proprioception, and centrifugation influence centripetal drift of gaze.</p>	<p>Develop methods to determine whether gaze-holding is impaired following spaceflight, why it fails in patients, and how it can be remedied.</p>	<p>Develop training countermeasures to reduce gaze-holding deficits.</p>
<p>SHELHAMER/ Context-Specificity and Other Approaches to Neurovestibular Adaptation</p>	<ul style="list-style-type: none"> • Re-entry & landing vertigo • Postlanding vertigo & visual instability 	<ul style="list-style-type: none"> • Monitoring and diagnosis (assessment & prediction) • Training 	<ul style="list-style-type: none"> • Human, 1-G sled and parabolic flight. • Animal (primate), sled 	<ul style="list-style-type: none"> • Determine context cues for VOR and saccade adaptation, and effects of torsional misalignment in 1-G and parabolic flight. • Determine whether context specific adaptation transfers between eye and limb movements 	<p>Determine whether saccadic or cyclovergence adaptation may be useful countermeasures.</p>	<p>Develop appropriate assessment methods and context specific adaptation training countermeasure.</p>

<p>WALL/ Advanced Techniques to Assess and Counter Gait Ataxia</p>	<ul style="list-style-type: none"> • Postlanding imbalance, vertigo & visual instability 	<ul style="list-style-type: none"> • Monitoring and Diagnosis (Prediction) • Training • Prosthesis 	<ul style="list-style-type: none"> • Human, walking on disturbance platforms and circular treadmill. • Tactile prosthesis. 	<p>Quantify body, head & gaze response to perturbed and circular walking.</p>	<ul style="list-style-type: none"> • Develop portable locomotion disturbance platform for use at JSC. • Develop tactile prosthesis and dynamic balance exercises 	<ul style="list-style-type: none"> • Define standards for postflight locomotion and gaze response to disturbance. • Evaluate effectiveness of tactile prosthesis and dynamic balance exercises
<p>YOUNG/ Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation</p>	<ul style="list-style-type: none"> • Vestibular & biomechanical effects of artificial gravity • Acute space motion sickness • Chronic space motion sickness 	<ul style="list-style-type: none"> • Monitoring and diagnosis (assessment & prediction) • Training • Environmental manipulation • Pharmacological agents 	<p>Human, short radius centrifuges and rotating chair.</p>	<p>Determine how context cues influence VOR, perception, and motion sickness adaptation, and which kind of conflict cues drive adaptation.</p>	<p>Determine optimal conditions for developing VOR and limb movement adaptation to Coriolis effects. Determine whether drugs affect Coriolis adaptation</p>	<p>In concert with other teams, develop short-radius, intermittent centrifugation countermeasure for muscle, bone, cardiovascular, and vestibular reconditioning.</p>

IV. TEAM ACCOMPLISHMENTS

During the past year, our programmatically significant major progress relative to amelioration of each of our seven risks has been:

- 1) Re-entry and landing vertigo: Dr. Oman's project has demonstrated a new virtual reality based technique for direct measurement of head movement contingent oscillopsia. Subjects adjust the amount of scene motion produced by active head movements until the scene appears perceptually stable (CRL 5). A geometric gain greater than one is consistently required for perceptual stability. The method can potentially be adapted to measure oscillopsia due to OTTR or G excess illusions. Dr. Reschke's project has shown that during eccentric gaze holding, the time constant of centripetal drift is dependent on the subject's gravitational position. (CRL 3). Data from five patients has been obtained and is under analysis. Hypergravic experiments are planned for this fall.
- 2) Acute space motion sickness: Dr. Shelhamer's team continued parabolic flight experiments investigating possible changes in ocular vertical alignment (skew) which might cause diplopia and contribute to space sickness. (CRL 2), and continued to refine his experiments on inducing context specific changes in saccade gain. Dr. Reschke began to evaluate the use of electronic shutter glasses as a means of reducing sensory conflict during motion sickness (CRL 4)
- 3) Postlanding vertigo and postural instability: In recent years, Dr. Wall's laboratory has developed a wearable prototype multi-axis vibrotactile balance aid suitable for use in walking experiments. With NSBRI support Dr. Wall investigated the use of a vibrotactile balance aid to recover from anterior-posterior balance disturbances while standing. Meanwhile, the portable balance disturber ("BALDER") developed by Dr. Wall is now in use at JSC in Dr. Bloomberg's laboratory. Dr. Bloomberg continued his studies of visual acuity during treadmill locomotion, in subjects with both normal vision and while wearing x 0.5 minifying lenses, and at distances of both 0.5 and 4 meters. They found that subjects are able to modify full-body segmental kinematics in order to reduce head perturbations. These locomotion and visual acuity assessment techniques will be evaluated by the NSBRI Integrated Team for use in the postflight Clinical Status Evaluation (CSE), with the goal of obtaining normative postflight locomotion data for use in assessment of emergency egress capability, fitness for return-to-duty, and efficacy of rehabilitation. (CRL7). Bloomberg's treadmill visual acuity test is also scheduled for an inflight evaluation (ULF-2, about 2005). Dr. Shelhamer investigated measurement of the gain of the oculomotor response to transient head motions (lateral heaves and fore-aft surges), which might form the basis of a tool for postflight assessment of otolith and cerebellar function.

- 4) Inflight spatial disorientation and frame of reference problems: Drs. Oman's project developed new methods to quantify the direction of the perceived vertical using the "shape-from-shading" illusion, useful for assessment of the effects of polarity and frame cues provided by visual scenes (useful for design of spacecraft interiors and workspaces). They demonstrated the feasibility of a virtual reality based 3D spatial memory training countermeasure, comparing the effectiveness of randomized vs. blocked presentations (CRL 6). They also showed that rat head direction cells exhibit a distinct "disorientation" response state, corresponding to Type II/III spatial disorientation in humans.
- 5) Chronic space motion sickness, Dr. Dornhofer's project demonstrated that the midlatency auditory evoked P50 potential (a measure of the ability to filter out familiar sensory stimuli) changes during motion sickness onset (CRL 5), that the benzodiazepine Lorazepam (1 mg, p.o.) was ineffective at preventing Coriolis induced motion sickness, as compared to promethazine.
- 6) Artificial Gravity: Drs. Young's project has shown that 75% of normal subjects can tolerate single axis head movements at 23 RPM, and that incrementing the RPM while adapting reduces symptoms. They demonstrated that motor adaptations are rapid, that VOR adaptation to Coriolis stimulation requires a visual scene, that the angular VOR time constant shortens, and that adaptation transfers to some degree across two different directions of centrifuge rotation, important since astronauts ideally should remain oriented and asymptomatic regardless of the orientation of their head with respect to the direction of the centrifuge velocity vector. (CRL 5). Dr. Young's team are the neurovestibular participants in an international multi-disciplinary AG/bedrest evaluation now being planned by NASA, ESA and CNES. (CRL 6) Dr. Stephen Moore received NSBRI postdoctoral fellowship support for research in AG.
- 7) Chronic vestibular function changes: Dr. Dornhofer's project has evaluated measurement of 3D eye movements and deviation of the subjective visual vertical during off axis angular rotation testing. They showed that the SVV in patients with unilateral lesions deviates ipsilaterally (CRL 5).

The neurovestibular team has maintained a strong record of scientific productivity. Since the start of phase 2 research in 2000 and the present, 133 papers, chapters and abstracts have appeared, including 47 peer reviewed journal articles (Listed in Appendix). (Between 1997 and 2000, the initial 3 projects had 55 publications, including 14 journal articles.) Nine papers were published together in a NSBRI neurovestibular special issue of the Journal of Vestibular Research (12(5,6) in October, 2003. Ten graduate students, one summer intern and 10 postdoctoral trainees participated in the research program.

One new project was initiated this summer to study "wobblies" postlanding disorientation among unlimited level aerobatic pilots. The goal is to determine whether the high negative G levels experienced by these pilots is creating canalithiasis and symptoms of benign paroxysmal positional vertigo (BPPV). Eventually the group will consider whether some

cases of postflight vertigo among astronauts might also be BPPV. The initial 1 year effort is being supported with Director's funds, and involves Drs. Oman, Clark, Solomon, and Wood from NSBRI and three extramural coinvestigators (Rupert/NAMRL, Lewis/MEEI, and Pohelman/EAA-IAC). Oman and Solomon attended the US National Championship, and were invited to give presentations to several groups of competitors about spatial disorientation and wobblies.

This summer a Bioastronautics Integrated Team began to form in the Neurovestibular area. Dr. Jonathan Clark is the participating JSC Flight Surgeon. Drs. Oman, B. Cohen, Wall, Bloomberg, Dornhofer, Zee, Reschke and Paloski are participating. Initial efforts are focusing on evaluation of techniques for postflight Clinical Status Exams. A dialog with Shuttle EMU manufacturer Hamilton Sundstrand has been initiated regarding possible ways to ameliorate the in-suit vomiting risk. This fall the team is working with a JSC Biomedical Sciences Management Team to further define the Critical Path risks and critical questions, and to prioritize them between and within disciplines. One significant change is that risks will be defined and prioritized for three different reference missions (1 year ISS, 1 month lunar, and 18 month+ planetary).

Other activities: The Neurovestibular Team PIs met in Portland, OR in October, and reviewed the 2002 Strategic Plan. Minor changes were incorporated into a 2003 version, which also conformed to the format used by other NSBRI teams, Dr. Oman participated in the NSBRI Cardiovascular Team's annual retreat. We informally advise several neurovestibular-related projects underway on other teams: (Ray Vestibular Autonomic project /Cardiovascular Team; Morin Vestibular effects on circadian/Chronobiology; Putchu Intranasal motion sickness drug administration/Smart Medicine.) We are also in frequent contact with the NASA JSC Human Factors Engineering program (Code SF). Dr. Mihriban Whitmore recently replaces Dr. Jennifer Blume as our HFE point of contact. Revisions to NASA Manned Systems Integration Standards 3000 are under discussion with JSC's Dr. Brian Peacock.

The current project portfolio collectively addresses some aspects of all seven critical path risks. Four projects (Bloomberg, Oman, Wall, Young) have relatively countermeasure techniques ready for evaluation by the Bioastronautics Integrated Team, and several other concepts are at the CRL 5 level. Cuts to the NSBRI budget during FY 2002 and FY2003 unfortunately impacted progress on several projects. There remain are significant gaps in the current program, partly due to the thematic distribution of proposals solicited by the most recent NSBRI research announcement (NSBRI 00-001). The team strategy for closing these gaps is described in our 2003 Team Strategic Plan. All seven project principal investigators submitted new or competitive renewal proposals to the July NSBRI solicitation (NRA-03-OBPR-04) for the next funding period.

APPENDIX. Publications of the Neurovestibular Adaptation Team (2000-2003)

(By project, including abstracts, papers in press, and manuscripts submitted through October, 2003).

Bloomberg, et al

1. Mulavara AP, Miller CA, Houser J, Richard JT, Bloomberg JJ (2001). Gaze stabilization during locomotion requires full body coordination. 25th Annual Meeting of the American Society of Biomechanics, San Diego, CA, August 7-12, 2001.
2. Bloomberg JJ, Mulavara AP, Kozlovskaya IB (2002). Reorganization of locomotor strategies after long-duration spaceflight. Annual Aerospace Medical Association Meeting, Montreal, May, 2002.
3. Bloomberg JJ, Mulavara AP, Kozlovskaya IB (2002). Adaptive reorganization of locomotor function after long-duration spaceflight. XII Conference on Space Biology and Aerospace Medicine, Moscow, June 10-14, 2002.
4. Mulavara AP, Bloomberg JJ (2002). Identifying head-trunk and lower limb contributions to gaze stabilization during locomotion. Sixth Symposium on the Role on the Vestibular Organs in the Exploration of Space, Portland, October, 2002.
5. Peters BT, Bloomberg, JJ (2002). Dynamic visual acuity during locomotion using far and near targets. Sixth Symposium on the Role on the Vestibular Organs in the Exploration of Space Portland, October, 2002.
6. Bloomberg JJ, Mulavara AP (2002). Understanding astronaut locomotor dysfunction after spaceflight: a sensorimotor perspective. Inter-Congress of the International Union of Anthropological and Ethnological Sciences, Tokyo, Japan, September 22-27, 2002.
7. Bloomberg JJ, Mulavara AP, Cohen HS (2003). Functional coordination of a full-body gaze control mechanisms elicited during locomotion. Space Biomedical Investigators Workshop, Galveston, Jan. 2003
8. Miller CA, Mulavara AP, Richards JT, Peters BT, Houser J, Marshburn AM, Bloomberg JJ (2003). Coordination of lower-limb joints during locomotion: the effects of vestibulo-ocular reflex adaptation. American Society of Biomechanics, Toledo, Oct. 2003.
9. Bloomberg JJ, Mulavara AP (2003). Changes in walking strategies after spaceflight. IEEE Engineering in Medicine and Biology Magazine, 22(2): 58-62.
10. Mulavara AP, Bloomberg JJ (2003). Identifying head-trunk and lower limb contributions to gaze stabilization during locomotion. Journal of Vestibular Research, 12: 255-270

Dornhoffer, et al

1. Dornhoffer J, Chelonis JJ, Blake D. (2003) Stimulation of the semicircular canals via the rotary chair as a means to test pharmacologic countermeasures for space motion sickness. (Submitted to Otolology & Neurotology)
2. Dornhoffer JL, Mamiya N, Bray P, Skinner RD, Garcia-Rill E. (2003) Effects of Rotation on the sleep state-dependent midlatency auditory evoked P50 potential in the human. *Journal of Vestibular Research* 12(5/6).
3. Homma Y, Homma Y, Teneud L, Skinner RD, Dornhoffer J, Williams DK, Garcia-Rill E. (2003) Effects of rotation on the P13 mid-latency auditory evoked potential in rat. (2003) *Journal of Vestibular Research* (in press).
4. Garcia-Rill E, Skinner RD, Clothier J, Dornhoffer J, Uc E, Fann A, Mamiya N. (2003) The sleep state-dependent midlatency auditory evoked P50 potential in various disorders. *Thalamus & Related Systems* 36:1-11.
5. Paule MG, Chelonis JJ, Blake DJ, Dornhoffer JL. (2003) Effects of Induced Motion Sickness and/or Drug Countermeasures on Working Memory in Humans. (Abstract), Society for Neuroscience 2003 Annual Meeting, New Orleans, Louisiana.
6. Pauwels G, Hoppenbrouwers M., Dornhoffer J., Van de Heyning P., Wuyts F (2003) Specific Space Motion Sickness Related Drugs Affect Only the Horizontal Semi Circular Canal Function But Not the Utricular Function (Abstract). Association for Research in Otolaryngology Midwinter Meeting, Daytona Beach, FL.
7. Dornhoffer J, Garcia-Rill E, Paule M, Van De Heyning P, et al. (2003) Pharmacological Countermeasures for Space Motion Sickness. (Abstract) Bioastronautics Investigators Workshop, Galveston, Texas, January 13-15.
8. Garcia-Rill E, Skinner RD. (2001) The sleep state-dependent P50 midlatency auditory evoked potential. In *Sleep Medicine* TL Lee-Chiong, MA Carskadon, MJ Sateia, eds, Philadelphia: Hanley & Belfus, p. 697-704
9. Wuyts FL, Pauwels G, Hoppenbrouwers GM, Dornhoffer J, Van de Heyning P. (2002) Pharmaceutical countermeasures for space motion sickness. (Abstract) The Role of the Vestibular Organs in the Exploration of Space. Portland, Oregon.
10. Wuyts F, Hoppenbrouwers M, Pauwels G, Van de Heyning P (2002) Unilateral otolith function testing - Is the utricular function additive ? (Abstract) 22nd Meeting of the Bárány Society, Seattle.
11. Pauwels G, Hoppenbrouwers M, Van de Heyning P, Dornhoffer J, Wuyts FL (2002) Pharmaceutical countermeasures for space motion sickness and their effect on the otolith and canals (Abstract). 22nd Meeting of the Bárány Society, Seattle, September 2002.
12. Dornhoffer J, Garcia-Rill E, Paule M. (2002) Clinical Investigation of Pharmacological Countermeasures for Space Motion Sickness (Abstract). Panel

Presentation at the Aerospace Medical Association 73rd Annual Scientific Meeting, Montreal, Canada.

Oman, et al

1. Bassett JP, Taube JS (2001) Neural correlates for angular head velocity in the rat dorsal tegmental nucleus. *Journal of Neuroscience* 21: 5740-5751
2. Bassett JP, Marcroft JL, Taube JS (2002) Lesions of the dorsal tegmental nucleus of the rat impair path integration performance on a food carrying task (Abstract). *Soc Neurosci Abstr* 28: 584.2
3. Bassett JP, Taube JS (2001) Lesions of the dorsal tegmental nucleus of the rat disrupt head direction cell activity in the anterior thalamus(Abstract). *Soc Neurosci Abstr* 27: 852.29.
4. Brown JE, Yates BJ, Taube JS (2003) Does the vestibular system contribute to head direction cell activity in the rat? *Physiology and Behavior* 77: 743-748.
5. Calton JL, Stackman RW, Goodridge JP, Archey WB, Dudchenko PA, Taube JS (2003). Hippocampal place cell instability following lesions of the head direction cell network. *Journal of Neuroscience* (submitted).
6. Calton JL, Taube JS (2001) Head direction cell activity following bilateral lesions of posterior parietal cortex (Abstract). *Soc Neurosci Abstr* 27: 537.
7. Calton JL, Tullman ML, Taube JS (2000) Head direction cell activity in the anterodorsal thalamus during upside-down locomotion (Abstract). *Soc Neurosci Abstr* 26: 983
8. Groen, E.L., Jenkin, H.L. & Howard, I.P. (2002) Perception of self tilt in a true and illusory vertical plane. *Perception* 31: 1477-90.
9. Harris LR, Jaekl P, Jenkin M. (2002) Perceptual stability during head movement (Abstract). Presented at Barany XXII, Seattle, USA Sept 26-29 *Journal of Vestibular Research* 11: 250 (B18.4).
10. Harris LR, Jenkin H, Dyde RT, Kaiserman J, Jenkin M (2002) Visual and vestibular cues in judging the direction of 'up' (Abstract). Barany XXII, Seattle, USA Sept 26-29 *Journal of Vestibular Research* 11: 307 (BP12.4).
11. Harris LR, Jenkin HL, Dyde RT, Kaiserman J, Jenkin M (2002). Relative role of visual and non-visual cues in judging the direction of 'up':experiments in the York tumbled room facility (Abstract). The Role of the Vestibular Organs in the Exploration of Space. Portland, USA. Sept 26-29 *Journal of Vestibular Research* 11: 325-6 (N6.6).
12. Howard IP, Jenkin HL, Hu G. (2000) Visually-induced reorientation illusions as a function of age. *Aviat Space Environ Med.*71: A87-91.
13. Howard IP, Hu G, and Zacher JE (2003) Visual orientation in a 90° mirror world. (Submitted)

14. Howard, I.P. and Hu, G. (2001) Visually induced reorientation illusions. *Perception*, 30, 583-600.
15. Jaekl P, Zikovitz DC, Jenkin MR, Jenkin HL, Zacher JE, Harris LR (2003) Gravity and perceptual stability during translational head movement on earth and in microgravity *Acta Astronautica* (submitted)
16. Jaekl P, Harris LR, Jenkin M (2002) The role of visual and vestibular cues in determining perceptual stability during head movement (Abstract). Presented at Vestibular Influences on Movement. Seattle, USA Sept 22-26 *Journal of Vestibular Research* 11: 197 (SP6.1).
17. Jaekl P, Jenkin M, Dyde RT, Harris LR (2003) Perceptual stability during active and passive head translation: variations with direction (Abstract) Vision Sciences Society annual meeting 2003.
18. Jaekl P, Jenkin M, Zacher J, Harris LR (2002) Gravity and perceptual stability during head movement (Abstract). The Role of the Vestibular Organs in the Exploration of Space. Portland, USA. Sept 26-29 *Journal of Vestibular Research* 11: 329-330 (N7.7) .
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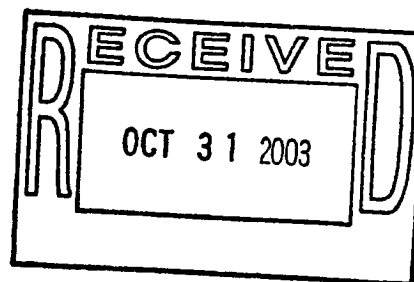


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I. ABSTRACT

Optimal human performance during space exploration requires the maintenance of all physiological systems, which in turn is dependent on adequate nutrition and physical fitness. The critical issues for nutrition are: (1) determining nutrient needs to meet modified requirements due to space flight stressors including microgravity; and (2) developing new strategies including use of functional foods, supplements, and timing of food intake relative to specific activities that will optimize human performance. Critical issues for physical fitness include: (1) development of appropriate aerobic and resistive exercises (mode, frequency, duration, intensity) and the appropriate balance for each to maintain aerobic capacity and muscle performance (as measured by strength and endurance) and (2) optimizing the appropriate timing of exercise programs with respect to food intake and other activities (e.g. extra vehicular activity; EVA). Since physical activity will, in part, determine nutrient needs, and the optimization of nutrient delivery will in part depend upon blood flow and muscle mass (which are affected by physical activity) these two disciplines need to be considered together. Examples of relevant risks that may be ameliorated by nutrition and physical activity interventions are: Reduced cardiovascular capacity; Loss of bone mineral density; Diminution of skeletal muscle function; Depressed immune response; Radiation enhanced development of cancer; Decrease in cognitive function; Alterations in sleep patterns; Psychosocial factors.

The nutrition and physical fitness program is new, becoming operational in 2001. It presently consists of three nutrition countermeasure projects (Lupton, Wolfe, and Tobin), a modeling project (Cabrera) and a new project which combines nutrition and physical fitness (Roubenoff). The original cornerstone project is the Wolfe bed rest study, which has seven "add on" projects and is fully integrated with the Tobin and Roubenoff projects. A critical finding from the Wolfe bed rest study was that nutrition may ameliorate muscle wasting but it does not preserve the loss of muscle strength. This reinforced the need for a combined nutrition/physical fitness intervention strategy, which was written into last year's request for proposals. In order to further maximize the effectiveness of the Wolfe bed rest study, we actively solicited proposals which would use the same overall design and nutritional intervention but would add a physical fitness component. We were very fortunate to receive and have funded an outstanding project by Dr. Roubenoff, which has now been fully coordinated with the Wolfe bed rest study. The Lupton project on the development of nutritional countermeasures to radiation enhanced colon cancer resulted in several discoveries: that exposure to 1Gy iron ions does, in fact, enhance preneoplastic markers of colon tumorigenesis, and that fish oil feeding and a fiber supplement can ameliorate this negative effect. Importantly, if rats are provided diets high in both fish oil and the fermentable fiber pectin, the enhanced tumorigenic effect seen with radiation exposure is no longer observed. The use of microarray technology to monitor changes in gene expression has identified key genes that are turned on (or off) as a result of carcinogen and/or radiation exposure. This technology can later be applied to humans. The Cabrera modeling project is now fully functional and he has established important collaborative efforts with members of other NSBRI teams. The Roubenoff project has just been initiated (September 1, 2003).

In summary, the Nutrition and Physical Fitness team has fully integrated its existing projects, expanded by the addition of small projects, and a new bed rest study which incorporates both nutrition and physical fitness. Optimal diet and physical activity protocols for space flight will impact every aspect of astronaut health and performance.

II. INTRODUCTION

The two major problems encountered in space that are currently addressed by the Nutrition and Physical Fitness Research Program are (1) Muscle Alterations and Atrophy and (2) radiation enhanced development of cancer. With respect to Muscle Alterations and Atrophy, the following are the Relevant risks (numbered in parentheses), found on the Critical Path Roadmap, that may be ameliorated by nutrition and physical activity interventions: Loss of Skeletal Muscle Mass, Strength, and/or Endurance (28); Inability to Perform Tasks Due to Motor Performance, Muscle Endurance, and Disruption in Structural and Functional Properties of Soft and Hard Connective Tissues of the Axial Skeleton (29); Inability to Sustain Muscle Performance Levels to Meet Demands of Performing Activities of Varying Intensities (30); Propensity to Develop Muscle Injury, Connective Tissue Dysfunction, and Bone Fracture Due to Deficiencies in Motor Skill, Muscle Strength and Muscular Fatigue (31); and Impact of Deficits in Skeletal Muscle Structure and Function on other Systems (32). Both appropriate nutrition and physical fitness can have a significant impact on muscle mass and strength. Nutrition is required to provide amino acids for muscle protein synthesis and energy for strength and endurance. Muscle protein synthesis is known to be depressed during space flight, due, in large part to muscular inactivity. This depression of protein synthesis is accompanied by an increase in protein degradation due to a moderate level of hypercortisolemia observed during space flight. Both aerobic and resistive exercise are also critical for proper muscle function. Aerobic exercise helps maintain blood flow to muscle so that nutrients can reach myocytes. Resistive exercise is key to maintaining muscle strength. Not only must energy expended on physical activity be balanced by appropriate food intake, the timing of exercise with respect to food ingestion impacts such important physiological effects as uptake of amino acids into muscle. Thus specific nutrients, ingested at the appropriate time, may help to maintain muscle mass. The relevant risks on the critical path addressed are 28-32 and the questions are 8.01, 8.02, 8.03, 8.04 and 8.05.

With respect to radiation enhanced development of cancer: The risks to personnel in space from naturally occurring radiations are generally considered to be the most serious limitation to human space missions and research in this area is now a top priority for NASA. Ionizing radiation results in the production of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radical, which are mutagenic and well documented to be carcinogenic in animals and humans. ROS accumulate with time, and it has been shown in a number of different systems that the greater the production of ROS, the higher the level of oxidative DNA damage. ROS can permanently damage nucleic acids inducing some 20 major oxidative DNA adducts some of which can go on to form tumors. Interestingly, diet may play an important role in removal of these DNA-adducted cells and thus protect against radiation-enhanced tumorigenesis. The NSBRI funded project of Lupton et al. uses diet as a countermeasure to selectively remove DNA-damaged cells from the colon by targeted apoptosis. Colon cancer is chosen for the model system as it is the second leading cause of death from cancer in the United States today, it strikes men and women equally, and it is the cancer most responsive to diet. The relevant risk on the critical path that is addressed is: Carcinogenesis Caused by Radiation (38) and the questions addressed are 10.02, 10.03, 10.05 and 10.09.

III. TEAM STRUCTURE & DESIGN

The Nutrition and Physical Fitness Team became operational in 2001. It presently consists of three nutrition countermeasure projects (Lupton, Wolfe, and Tobin), and two projects that combine nutrition and physical fitness (Cabrera and Roubenoff).

Specifically, ***Nutritional Countermeasures to Radiation Exposure*** JR Lupton, PI, Texas A&M University, is testing the hypothesis that a particular diet intervention (an n-3 lipid and fermentable fiber combination) in rats should protect against radiation-enhanced colon cancer by targeting DNA damaged cells for apoptotic removal. It is directed to Goal 5 of the strategic plan : Reduce Risk of Radiation Enhanced Development of Cancer and will also contribute to Goal 1: Reduce Risk of Suboptimal Nutritional Status. Rats receive one of four diets, are exposed to heavy iron radiation at Brookhaven National Laboratory and are injected (or not) with a colon specific carcinogen. A variety of measurements are taken at three stages of the tumorigenic process (initiation, promotion, and final tumor development). This project also has a noninvasive component of monitoring changes in gene expression over time as a result of radiation and carcinogen exposure using microarray technology. If validated in rats, the diets and techniques can be modified for future studies in humans. This noninvasive technology is also directed at Goal 12: Develop noninvasive techniques for assessing the effectiveness of diet and physical fitness interventions.

Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress, R. R. Wolfe, PI, University of Texas Medical Branch at Galveston, is testing an amino acid supplement designed to ameliorate muscle wasting induced by stress-and microgravity-induced depression of protein synthesis in a bed rest study. The study consists of 12 individuals with or without consumption of the supplement in a 30-day bed rest trial. A unique feature of this study is the use of a cortisol infusion at two times during the intervention period to mimic (in part) the documented elevated cortisol levels during space flight. Although primarily targeted to Goal 3: Reduce Risk of Diminution of Skeletal Muscle Function, we have considered this bed rest study to be our cornerstone project and have added a large number of ancillary grants which use the bed rest model and the nutritional intervention to address issues related to other goals. These “add on” projects will be discussed further in reference to Goal 14: Integrate Research and Analysis. To summarize here, separate projects working off of the Wolfe bed rest study are targeted to: Goal 1: Reduce Risk of Suboptimal Nutritional Status; Goal 2: Reduce Risk of Suboptimal Physical Fitness; Goal 3: Reduce Risk of Diminution of Skeletal Muscle Function; Goal 9: Reduce Risk of Depressed Immune Function; Goal 10: Reduce Risk of Loss of Bone Mineral Density; and Goal 14: Integrate Research and Analysis.

Nutritional Modulation of Pancreatic Endocrine Function in Microgravity B. W. Tobin, PI, Mercer University School of Medicine, is determining amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. Dr. Tobin uses human pancreatic islet cells cultured on static plates or in a high aspect ratio vessel (HARV) designed to replicate some of the conditions of microgravity. The goal of this research project is to determine how different physiological conditions, characterized by over or underexpression of certain hormones, affect insulin secretion and to develop an amino acid combination that will optimize this secretion. In becoming part of the Nutrition and Physical Fitness Team, Dr. Tobin has added a myocyte

culture model to determine the effect of maximizing insulin secretion on muscle cell response. In addition to targeting Goal 1 (as do all nutrition based projects), this project specifically addresses Goal 3: Reduce Risk of Diminution of Skeletal Muscle Function, since uptake of amino acids into muscle is governed by insulin. Optimal insulin synthesis, should maximize uptake of amino acids into muscle and thus enhance muscle protein synthesis. The other goal targeted by this research project is Goal 14: Integrate Research and Analysis. This goal will be described more fully later under Goal 14, but briefly stated, Tobin's project is using the amino acid levels found in blood of subjects from Wolfe's bed rest study who have received his amino acid supplement. Thus, there is integration between these two projects.

Metabolic Adaptations of Skeletal Muscle to Training/Detraining. A Systems Model M. E. Cabrera, PI, Case Western Reserve University, uses mathematical modeling to perform quantitative predictions of work capacity after periods of training/detraining. These models and predictive equations are based on data from animal and human studies and will provide a framework for quantitative understanding of the skeletal muscle metabolic adaptations to periods of training and detraining. Part of the database used in these predictive equations is nutritional information or metabolic status. Therefore, this research program serves to integrate both nutrition and physical fitness and targets goals 1,2, and 3 as well as goal 14, the integration.

Timed Feeding and Resistance Training To Prevent Muscle Atrophy, Ronenn Roubenoff, M.D. PI, Tufts University combines both nutrition and physical fitness in addition to the timing between the two. Our team has as its first priority combining nutrition and physical fitness to ameliorate muscle wasting. This project was just funded, and recruitment to the bed rest study began September 1, 2003. The project has the same experimental design and the same amino acid supplement as the Wolfe bed rest study so that results can be compared. The amino acid supplement is combined with an exercise component and a second variable is the timing of the supplement intake with respect to exercise. This project in combination with the Wolfe project should provide definitive (ground based) answers to whether exercise when added to the nutritional supplement at the right time can ameliorate muscle loss and the diminution of muscle strength. This research program serves to integrate both nutrition and physical fitness and targets goals 1,2, and 3 as well as goal 14, the integration.

We anticipate that the ground based research summarized above, combined with future projects (discussed below) will eventually result in three fundamental countermeasure strategies to provide optimal nutrition and physical fitness, which in turn will ameliorate the risks as elucidated on the Critical Path. These general, broad-based strategies are summarized below.

- 1) Development of the rationale and mechanistic justification for a combination of traditional and targeted functional foods which are highly palatable and designed to minimize the risks on the Critical Path, without negatively impacting either food intake or other risks for which they may not be specifically targeted. For example, an amino acid supplement designed to enhance protein synthesis should not depress immune response or negatively impact bone health. A coordinated effort at the team level is required to achieve this goal. Some of these foods/supplements will be general to meet the nutrient requirements for all individuals in space. Others may need to be task specific, e.g. time release energy foods for prolonged activity without additional food intake such as may be experienced during Extravehicular Activity (EVA).

Although it is not the goal of the nutrition team to develop the foods and supplements, it is a team goal to determine the requirements for what should be in those foods/supplements.

2) Development of an exercise protocol, and the appropriate equipment to maximize both muscle strength, lean body mass, bone strength, and aerobic capacity. Studies will be designed to determine the optimal as well as minimal prescription for frequency, duration, and intensity of the exercise countermeasure to obtain the most time efficient method to maintain muscle and cardiovascular capacity. Traditionally, this prescription is considered to involve two types of exercise protocols (resistance training and aerobic exercise), but where possible, their integration should be a priority. The overall intention of the physical fitness program is to produce the most physically fit individual (from both a strength and aerobic viewpoint) in the least amount of time. Since exercise takes time from other tasks and also requires energy input, which means greater food intake, accomplishing this task will have many benefits. In addition, the Nutrition and Physical Fitness Team is aware that different forms of preflight and in-flight physical exercise are a major countermeasure thrust for the Muscle Team and will work with the Muscle Team to coordinate and maximize the effectiveness of our collective programs to address shared goals.

3) Development of a strategy of timing of food intake with respect to physical activity. This countermeasure plan will be key to the overall health of individuals in space. Often overlooked, when one eats with respect to when one exercises has important consequences for overall utilization of nutrients and for human performance. The current recommendations for food intake timing with respect to exercise as practiced in flight are not based on strong scientific studies. A scientific basis for the timing of food intake and exercise prescriptions is needed. For example, R. Wolfe has shown in human studies, that providing amino acids prior to rather than after an exercise bout will enhance protein synthesis by up to three fold. The appropriate combination of foods or new functional foods with time release components could provide a certain level of blood glucose over extended periods of time so that exercise or other tasks such as EVA could be performed without stopping to eat. The current Roubenoff project should help resolve some of the issues with respect to timing of food intake with respect to exercise.

Keeping these three overarching countermeasure strategies in mind, the following is a discussion of the current status and future plans of the program with respect to the ten risk-based and four non-risk based goals defined in our Strategic Plan. As noted above, goals 1 and 2 are considered central to all of the other goals. They are (1) Reduce Risk of Suboptimal Nutritional Status and (2) Reduce Risk of Suboptimal Physical Fitness. With respect to Goal 1, designing optimal diets for individuals in space is not just taking the recommended dietary reference intake values (DRIs) developed for Americans and Canadians and modifying them for microgravity conditions. Optimal nutritional status for maximal performance both in space and for optimal health after space flight has to be more than meeting minimal RDI requirements. One needs to ask the question: "Optimal for what?" and in this case it is for maintaining muscle strength, bone mass, immune function, etc. This fact means that all of the nutrition projects (Lupton, Wolfe, Tobin, and aspects of Roubenoff) are addressing various aspects of what would represent optimal nutrition – Lupton from a view towards protecting against radiation-enhanced cancer; Wolfe, Tobin and Roubenoff from the viewpoint of maintaining muscle mass through amino acid uptake into muscle and appropriate insulin response.

With respect to Goal 2 (Reduce Risk of Suboptimal Physical Fitness), again, development of successful countermeasures to meet this goal underlies all of the other goals. In many ways, there are already potential countermeasures at a high stage of development (exercise equipment and protocols) and with further relatively simple studies, we could have countermeasures in place within a rapid time frame. The Nutrition and Physical Fitness Team sees Goal 2 as being very practically oriented, rather than at the level of basic science. Protocols to be tested need to be ones that can be used in space and should aim towards the maximum benefits in the shortest amount of time. The funding of the Roubenoff project is very helpful towards achieving this goal, as it integrates exercise with diet and uses the same protocol as the Wolfe bed rest study.

Goal 3 (Reduce Risk of Diminution of Skeletal Muscle Function), is the primary research focus of the current program and four out of the five projects address this goal (Wolfe, Tobin, Cabrera, Roubenoff). The problem addressed is that muscular inactivity leads to decreased protein synthesis. This problem is compounded by the fact that stress (mediated by moderate hypercortisolemia) leads to increased protein breakdown. The combined effect of decreased synthesis and increased breakdown results in loss of skeletal muscle mass, which leads to loss of muscle strength. This compromises crew capabilities, including EVA or potential emergency egress. Countermeasures to these risks include an amino acid supplement designed to enhance protein synthesis (Wolfe bed rest study) which should also enhance insulin secretion and thus amino acid uptake into muscle and muscle synthesis (Tobin, insulin secretion). The timing of this amino acid supplement with respect to an exercise protocol (Roubenoff) should be the maximally effective countermeasure. This dietary countermeasure, combined with an appropriate physical fitness intervention should help maintain muscle strength and aerobic capacity, positively affecting both muscle strength and uptake of amino acids into muscle for protein synthesis. In the future, the Cabrera modeling project will take data from the Wolfe, Tobin and Roubenoff programs and combine it with existing data from previously conducted research, to develop equations that will predict for work capacity after periods of training/detraining. Progress towards achieving this goal is advancing, and the goal is adequately addressed by current research projects. In addition, as we more fully integrate with the Muscle Team (See Goal #14), our combined strengths in this area position us to advance rapidly through phases of countermeasure development.

Goal 5 (Reduce Risk of Radiation Enhanced Development of Cancer) is currently being addressed by the Lupton project. Risks to personnel in space from radiation exposure are considered to be a tier one problem by NASA. A primary risk of radiation exposure is later cancer development. Of all the cancers, colon cancer is the second leading cause of death from cancer in the United States today. It strikes men and women equally. On the positive side, it is the cancer most amenable to diet intervention. Thus, studying mechanisms by which we can protect against the development of this cancer with respect to previous radiation exposure is important.

Our team also has non risk-based goals which are at various levels of development at this time. For example, Goal 12: Develop Noninvasive Technologies for Assessing the Effectiveness of Diet and Physical Fitness Interventions is in its infancy. One important aspect of the Lupton project is the use of microarray technology on mRNA from fecal material to see which genes are turned on or off during particular diet interventions, which ones are affected by radiation

exposure and how these gene array patterns predict for a variety of endpoints. This patented technique is well developed in the rat, and the plan is to later apply it to humans. As noted previously, diet and physical fitness interventions lend themselves very well to Earth-based applications (Goal 13). In particular, we envision a protein supplement that will enhance amino acid uptake into muscle and muscle protein synthesis as a result of the Wolfe bed rest study. This supplement will also be useful for individuals on earth who have muscle wasting due to a variety of causes. We also envision a supplement of omega 3 fatty acids combined with pectin (a fermentable fiber) which may protect against both oxidative and alkylation damage to colonic DNA. With colon cancer the second leading cause of death from cancer in the US today, such a supplement could prove to be very beneficial.

Goal 14: Integrate Research and Analysis has already been a major part of the Nutrition and Physical Fitness Team. This goal includes efforts to enhance the interaction of individual Nutrition and Physical Fitness Team investigators a) among the current team's infrastructure, b) among investigators within other teams (eg. Muscle, Radiation), and c) with investigators not formally associated with the NSBRI. The activities will allow us to greatly expand the resources of NSBRI and the ability to tackle the risks of space travel. Table 1 summarizes our current efforts at integration. In addition to strong collaborations within our team, a few examples of integration of the Nutrition and Physical Fitness Team with other teams or researchers outside of the NSBRI are as follows: Lupton is collaborating with Judex (bone team) in supplying rat hind limbs from irradiated rats on different diets. The Wolfe bed rest study is a true collaborative effort with the following investigators/projects: P. Uchakin, Mercer University, testing the hypothesis that stress during inactivity alters the balance between cell-mediated and humoral immunity; S. M. Smith, NASA, JSC, The effect of bed rest and amino acid supplementation on bone markers of calcium metabolism; R.R. Fitts, Marquette University, The effect of prolonged bed rest and amino acid supplementation on muscle fiber function; R Stowe, UTMB, Effects of prolonged bed rest on herpesvirus-specific immunity. Similar measurements to the Stowe bed rest study are also being performed on the Shuttle and ISS crewmembers and so the Stowe study would serve to complement in-flight work. Additional collaborative projects with the Wolfe study include: T.P. Stein, UMD-NJ, Does bed rest + hypercortisolemia lead to increased oxidative stress during the recovery phase?; H W Lane, NASA, JSC, The effect of bed rest and amino acid supplementation on muscle energy production during exercise. In addition to collaborative projects, several people who were not directly funded with NSBRI grants have become an active part of our team. They include Helen Lane and Scott Smith from NASA/JSC in the area of nutrition, Don Hagan, also NASA/JSC for physical fitness, and Michelle Perchonok, NASA/JSC for food science. These close ties to NASA enable the Nutrition and Physical Fitness Team to be up to date on the most recent countermeasure approaches to addressing nutrition and physical fitness related risks.

IV. TEAM ACCOMPLISHMENTS

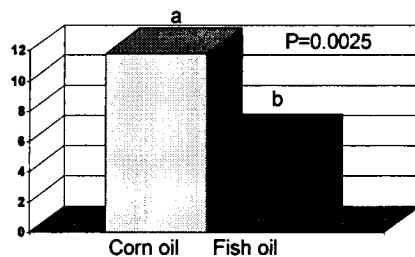
During the past year excellent progress has been made on each of the 4 funded projects and the 5th project has recently been initiated. Specifically:

Nutritional Countermeasures to Radiation Exposure, JR Lupton, PI, Texas A&M University.

During the past year almost all of the animal work has been completed, and all of the irradiation at Brookhaven National Laboratory is complete. Of the total of 560 rats, all of the irradiated rats

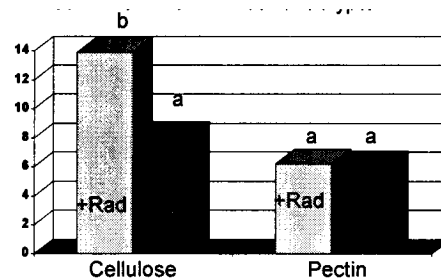
plus 3/4 of the non-irradiated rats have been terminated. The program is designed in such a way that all of the analyses are complete in the third year (the project is now in its second year) and thus statistically significant data are not available in all areas. However, at this time the project has shown that: One dose of radiation (Fe ions) 1 Gy in combination with a colon specific carcinogen results in a greater number of aberrant crypts (precursors to colon tumors) and a greater number and variety of tumors than does injection with the carcinogen alone. Importantly, a combination of fish oil and pectin can negate the additive effect of the radiation.

Mean #ACF with > 3 Aberrant crypts



Rats fed fish oil (n=20) had a lower number of ACF with more than 3 aberrant crypts in a cluster than did rats fed corn oil (n=20). From (Lupton et al., 2003, Popovic et al., 2003a)

Mean #ACF with > 3 Aberrant crypts



Providing pectin in the diet reduced the radiation-enhancing effects on ACF development to the same level as rats not receiving radiation.

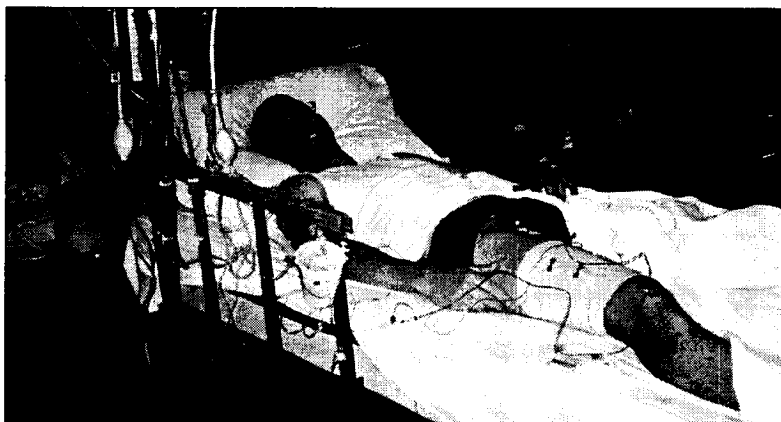
Currently mRNA from the same rats in this experiment has been collected and appropriately stored and will be subjected to PCR and microarray analysis to determine which genes were up or down regulated by the radiation and carcinogen treatments.

The project to date has resulted in 3 published manuscripts; 2 *In Press*, 2 submitted and 13 abstracts. To summarize, this project is proceeding on schedule, and the initial results confirm the hypothesis that radiation exposure in addition to chemical carcinogen exposure, enhances colon tumor development, and that diet can act as an effective countermeasure.

Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress, R. R. Wolfe, PI, University of Texas Medical Branch at Galveston.

The intervention phase of this research project has been completed ahead of schedule. The first subject is shown below.

Subject in Wolfe bed rest study.



Most importantly we have chosen the bed rest study as the cornerstone of our program, and as such have requested and received funding for a number of important additional projects which use samples from this study. The following are descriptions of “add ons” to the Wolfe bed rest study. Data collection is also complete from all of these studies.

1. Helen Lane, Ph.D.

NASA Johnson Space Center
Houston, TX

Title: Response of oxidative capacity in skeletal muscle to prolonged bed rest.

The NSBRI project evaluates a nutritional countermeasure, protein/amino acid supplementation, on leg muscle performance after a month of bed rest. As part of this overall project, Dr. Helen Lane is leading the NMR spectroscopy for determination of oxidative capacity of calf muscle before, during, and after exercise. Phosphorus NMR spectroscopy is a technique to continuously follow high energy metabolism in a localized tissue by determination, using P31, of levels of inorganic phosphate, phosphocreatine, and ATP along with calculation of muscle pH. This technique also allows for determination of creatine kinase reaction at rest and during exercise in skeletal muscles. Creatine kinase catalyses the reversible transfer of high-energy phosphate groups of phosphocreatine to and from adenosine diphosphate. Muscle countermeasures such as training programs changed these parameters both in the level of phosphocreatine as well as the time to recovery of phosphocreatine after fatiguing exercise. The plan is several fold: First, we need to develop the hardware (exercise device, interface for quantification, and other analytical efforts such as precision and reproducibility) and the software interface to determine the oxidative capacity of calf muscle with phosphorus NMR spectroscopy. Next, we will examine the difference in these parameters before and after 28d of bed rest with and without the countermeasure. Our hypothesis is that in subjects without the countermeasure phosphocreatine will be lower at rest and decrease more during exercise than those subjects with a countermeasure. Furthermore, the regeneration of phosphocreatine will be faster in those who have the countermeasure. These measures will be correlated with muscle function (strength measures) and single fiber analyses performed by Bob Fitts (as a separate add-on project). Thus, we will not only determine the effect of bed rest on muscle oxidative capacity, but the relation of oxidative capacity to muscle function. This project is being conducted at no additional cost to NSBRI.

2. Scott M. Smith, Ph.D. (Also a collaborator on the Tobin project)

Research Nutritionist
NASA Johnson Space Center
Houston, TX

Title: Markers of bone and calcium metabolism

Dr. Scott M. Smith's supplement to the Wolfe project is designed to measure markers of bone and calcium metabolism in the bed rest subjects. These include serum osteocalcin and bone-specific alkaline phosphatase, and urinary collagen crosslinks. Osteocalcin and Bone-Specific

Alkaline Phosphatase are both bone formation markers. The collagen crosslinks are bone resorption markers, and represent a family of compounds which include n-telopeptide, pyridinoline, and deoxypyridinoline, among others. Their analyses will tell us if the Wolfe countermeasure, obviously aimed at muscle tissue, has any impact on the bone side of the musculoskeletal system. At present Dr. Smith has approximately 1/3 of the samples, but is awaiting analysis until after all samples are collected, to analyze these in one batch.

3. Raymond P. Stowe

Assistant Professor (Research), Pathology
University of Texas Medical Branch
Galveston, TX

Title: Changes in antiviral immunity during bed rest

Dr. Raymond P. Stowe's project measures changes in antiviral immunity. These measurements are also being performed on the Shuttle and ISS crewmembers through his NASA grant (Epstein-Barr virus flight grant). These include measurement of viral load in blood, urine, and saliva samples using molecular methods. In addition, he will measure virus-specific immunity by measuring anti-viral antibody titers and antigen (virus)-specific T-cells to correlate with viral load.

4. Brian W. Tobin, Ph.D.

Associate Professor
Mercer University School of Medicine
Macon, GA

Title: Blood amino acid levels from Wolfe project for use in Tobin project

In order to test the same protocols in several model systems, the amino acid blood levels produced by the Wolfe supplement have been quantitated and will be used as one of the interventions in the Tobin protocol. This is at no added expense to NSBRI.

5. Peter N. Uchakin, Ph.D. (Also a lead investigator on the Tobin project)

Lead Research Scientist
Division of Basic Medical Sciences
Mercer University School of Medicine
Macon, Georgia

Title: The effect of bed rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines

Dr. Peter N. Uchakin is investigating the effects of bed-rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines such as IL-1, IL-6 and IL-10. In addition, Dr. Uchakin will assess immunocyte distribution in whole blood.

6. Robert Fitts, Ph.D.

Marquette University
Milwaukee, WI

Title: Single muscle fiber function in response to bed rest and nutritional intervention.

Investigation of the contractile properties and force of single muscle fibers of the soleus (predominantly Type 1) and vastus lateralis (predominantly mixed) in response to prolonged bed rest with and without the nutritional intervention.

7. T.P. Stein, Ph.D.
Professor of Surgery
UMDNJ-SOM
Stratford, NJ

Title: Markers of oxidative stress

Oxidative damage from free radicals to DNA and lipids has been implicated in the etiology of a wide variety of chronic diseases and acute pathological states. Dr. Stein and his colleagues had the opportunity to obtain data on the question of whether space flight has any effect on the oxidative status of astronauts. They measured the urinary excretion of 8-iso-PGF_{2a} and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) on 6 subjects (2 US astronauts and 4 Russian cosmonauts) before, during and after long duration space flight on the Russian space station MIR. The urinary excretion of the isoprostane 8-iso-PGF_{2a} and 8-OH dG are markers for oxidative damage to lipids and DNA respectively.

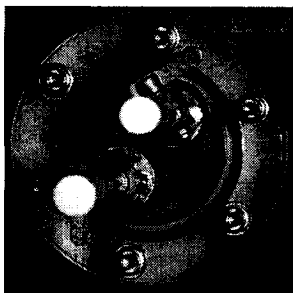
There was a trend towards an increase in 8-OH dG excretion in flight. Both 8-iso-PGF_{2a} and 8-OH dG excretion were double post flight indicating that oxidative stress was double. The increase persisted for the two-week observation period. The level was akin to smoking a pack of cigarettes a day. The increased oxidative stress damage post flight most likely reflects impaired endogenous anti-oxidant defenses. (Subjects took vitamin capsules throughout the study period). The down regulation of protein metabolism that occurred on MIR could cause some loss of protein-based antioxidant systems. The likely causes of the compromised protein metabolism are: (i) the combination of reductive remodeling in response to the loss of load and the chronic under-nutrition that occurred on MIR and (ii) competition post flight for amino acids between synthesis of defense related proteins and repleting muscle leading to sub-optimal availability of host defense mechanisms.

The problem being addressed is how to study this problem on the ground. The need is for an acceptable ground based model. At present there is no such model. The objectives of this supplementary project are therefore to determine whether cortisol administration to bed rest subjects reproduces the flight situation. Given a model, counter-measures can be explored. Thus the hypothesis being tested is that bed rest plus cortisol reproduces the flight result. Dr. Stein will use the urinary excretion of the isoprostane 8-iso-PGF_{2a} and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) to assess oxidative damage to lipids and DNA respectively in the urines from Dr. Wolfe's study.

In summary, the Wolfe/Ferrando bed rest study intervention phase was completed ahead of schedule and data from the ancillary projects are now being analyzed. The major findings are that essential amino acid supplementation stimulates muscle protein synthesis, results in maintenance of lean body mass, and ameliorates loss of muscle strength although a loss of strength is still evident.

Nutritional Modulation of Pancreatic Endocrine Function in Microgravity B. W. Tobin, PI, Mercer University School of Medicine, is determining amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. In addition, he has modified his protocol to include addressing these effects on human myocytes isolated from the same cadavers as the pancreatic islet cells. Islets or myocytes are cultured in “microgravity” or static plate controls. The HARV rotates at 10 RPM, and islets are elliptical orbit-suspended in media. Measurements are made of medium glucose, lactate, hormones and amino acids. Specific aims of this project are (1) to assess the effect of a microgravity model system on basal amino acid requirements and endocrine secretory function in human islets of Langerhans and (2) to determine human islet endocrine function while testing amino acid countermeasures. Results from the first set of studies illustrate (1) decreased glucose utilization, (2) enhanced insulin secretion, (3) increased utilization of cysteine, and (4) increased production of ornithine, presumably from arginine. Dr. Tobin is also actively collaborating with Wolfe/Ferrando and in fact Peter Uchakin (an investigator on the Tobin project) is now working with Wolfe/Ferrando on site (see the Wolfe/Ferrando summary above). In the past year he established a collaboration with R. Walzem (Texas A&M University) to measure lipid metabolites in pancreatic islet cell systems. Dr. Tobin’s group presented at the 2004 International Pancreas and Islet Transplantation Meetings which resulted in the recommendation that catecholamines be used as “potential immune enhancers for Edmonton Canada clinical islet trials”. The most significant findings from this project to date are: that pancreatic cells exposed to simulated microgravity conditions have a lower need for glucose, and a greater utilization of cysteine and phospholipids than their traditionally cultured counterparts.

High Aspect Ratio Vessel (HARV) used
To mimic microgravity conditions for
Culturing myocytes and pancreatic islet
Cells



Metabolic Adaptations of Skeletal Muscle to Training/Detraining. A Systems Model M. E. Cabrera, PI, Case Western Reserve University, uses mathematical modeling to perform quantitative predictions of work capacity after periods of training/detraining. These models and predictive equations are based on data from animal and human studies and will provide a framework for quantitative understanding of the skeletal muscle metabolic adaptations to periods of training and detraining. Part of the database used in these predictive equations is nutritional information or metabolic status. Therefore, this research program serves to integrate both nutrition and physical fitness and targets goals 1,2, and 3 as well as goal 14, the integration. Dr. Cabrera is in the beginning of the third year of funding for this grant. During that past year he continued the development and implementation of the computational model of skeletal muscle metabolism that integrates cellular, tissue, and whole body data and that incorporates specific parameters which have been identified as playing a major role in the responses to training and detraining such as muscle mass and enzyme activities. Computer simulations of responses to moderate exercise were performed on three muscle models representing different states: (a) normal sedentary subject, (b) trained subject, and (c) detrained subject. Then, they contrasted the exercise responses resulting from the model of a “trained muscle” to those from the model of a “detrained muscle”. Dr. Cabrera has established important networking relationships with other teams within the NSBRI. Specifically he has submitted two proposals to the recent request for proposals with Dr. Soller (Smart Medical Systems) Dr. Coolahan (Cardiovascular Alterations) and Dr. Kushmerich (Muscle Team).

Timed Feeding and Resistance Training To Prevent Muscle Atrophy, Ronenn Roubenoff, M.D. PI, Tufts University combines both nutrition and physical fitness in addition to the timing between the two. As soon as we knew that this project was funded we had a meeting at NASA/JSC to fully integrate the project with the Wolfe bed rest study and to assure that the exercise protocols were compatible with what NASA understands as being possible to conduct in space. The PI has just received his IRB approval and has begun recruiting for the bed rest study. They have also submitted an IRB to look at immune function effects of bed rest at no additional cost to the grant. Further, they are discussing with investigators at NASA/JSC the possibility of adding a calcium metabolism substudy to support efforts being discontinued at NASA. This project addresses critical path risks 28-32 (Loss of muscle mass, strength and/or endurance and the consequences) and questions 8.01, 8.02, 8.03, 8.04 and 8.05. It uses the same diet intervention as the Wolfe bed rest study combined with resistance training.

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**National Space Biomedical Research Institute
NUTRITION, PHYSICAL FITNESS AND REHABILITATION PROGRAM**

Table 1. Integration Activities

	CABRERA Metabolic Adaptations of Skeletal Muscle to Training/Detraining. A Systems Model	LUPTON Nutritional Countermeasures to Radiation Exposure	TOBIN Nutritional Modulation of Pancreatic Endocrine Function in Microgravity	WOLFE/Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress	ROUBENOFF Timed Feeding and Resistance Training To Prevent Muscle Atrophy,
Internal Communication	Entire team at meetings and telecons	Entire team at meetings and telecons	Entire team at meetings and telecons	Entire team at meetings and telecons. P. Uchakin from Tobin project is now situated in Wolfe lab.	Entire team at meetings and telecons
Integrated Experiment Development	Integrate existing knowledge into computational models of muscle metabolism using data from nutrition and physical fitness, the literature, and the muscle team	Uses the same strain of rat and the same radiation protocol at Brookhaven National Lab as does the Dicello rat long term studies (Radiation Team). Thus results can be compared.	Tobin has redesigned his research protocol as a result of talks with team members to now include myocyte cultures. He is also using blood from Wolfe study to determine levels of amino acids to use in his cell culture system. P. Uchakin, a co-investigator with Tobin, is now collaborating directly with Wolfe	This bed rest study is highly integrated with a variety of projects. The same amino acid supplement is tested in the Tobin project and we plan to have it tested in the Baldwin rat model (Muscle Team).	This bed rest study is integrated with the Wolfe bed rest study in that it uses the same experimental protocol and tests the same amino acid supplement. In addition it adds an exercise protocol and timing between supplement intake and exercise.
Sample Sharing	Data from Wolfe, Lupton, Schneider, Baldwin	Tobin will use pancreas from Lupton rats. S. Judex (bone team)	Will use pancreas from Lupton, will provide pancreatic	There are currently seven "add on" projects to the bed rest study . See discussion of Goal 14 in this	This study has not yet begun.

	(muscle team) and others from muscle team	will use rat hindlimbs, H. Hogan, TAMU, will also use bones.	cells to R Walzem, TAMU, for lipid analysis	report.	
Synergistic Studies of Opportunity	Aerobic and resistance exercise training with humans, and applying this to his model	Planned integration with A. Kennedy of radiation team for potential cross-use of each other's diet interventions	Future goal is to fly the HARV with pancreatic cells in space to determine how well the results mimic his system on earth	This study maximally capitalizes on synergistic opportunities which have arisen in the course of meetings and telecons to expand the program with "add on" grants.	The study will also investigate the potential for urinary loss of calcium as shown in the Wolfe study, to see if this effect is ameliorated with exercise.
Development of Computer Model of Integrated Human Function	This is exactly what the project is and should be an integrating force for the entire team.	Data from this project will be used by Cabrera for his prediction equations.	Currently this research is all ex vivo and not suitable for the integrated function component which we are only doing in animals and humans	Data from this project will be used by Cabrera for his prediction equations	Data will also be used by Cabrera for his prediction equations.



National Space Biomedical Research Institute

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A handwritten signature in black ink, appearing to read "John F. Dicello". The signature is fluid and cursive, with a large loop at the end.

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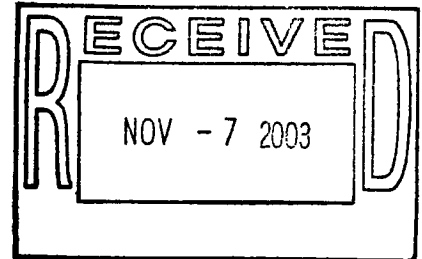
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ABSTRACT

The four newer projects of Drs. Vazquez, Kennedy, and Chang successfully completed irradiations with protons during the previous year at the Loma Linda University Medical Center and with energetic heavy ions at the Brookhaven National Laboratory. These PIs have been analyzing the results, some of which are presented in this report, and two of the PIs participated in the most recent accelerator run at BNL. The group's activities represent a major fraction of the total research for Radiation Health at the Brookhaven NASA facility.

Most recently, Dr. Ann Kennedy's group has compared the efficiency of four types of radiation in inducing oxidative stress in cultured cells and has identified several candidate agents that are highly effective in preventing radiation induced oxidative stress in vitro.

Dr. Chang's analysis of mutation frequency (MF) in the lacZ reporter transgene of mice exposed to acute doses of Gy proton irradiation showed that spontaneous lacZ MF in the control animals is tissue specific (spleen > brain).

Dr. Vazquez has exposed human neural precursor cells (NT2) and rodent glial progenitor cells (CG4) to acute doses of 0.1 to 6 Gy of heavy ions (Fe and Si, BNL-8) and gamma irradiation. Apoptosis induction as a function of time post irradiation was measured, with a dose-dependent peak induction of up to eight-fold above control levels after heavy ion, proton and gamma exposures. Estimated RBE's for apoptosis are the following Fe: 6, Si: 4 and protons: 1. Apoptosis pathways were examined at doses as low as 0.25 Gy.

In parallel with the in-vitro studies, 498 C57Bl/6 male mice were exposed to 1 GeV/n Fe ions at AGS and gamma rays. Monthly evaluation of the effects of heavy ion and gamma radiation on memory was carried out and monthly monitoring of cocaine-induced locomotor activity of heavy ion and gamma-irradiated mice was performed.

The two older projects previously were instructed by the NSBRI, in accordance with the recommendation of the NSBRI External Advisory Council, to concentrate on rats previously exposed and not to irradiate any further animals. All of the exposed animals have been successfully followed to death, and the breast tissues archived. The groups are presently processing the samples and completing the tissue analyses. Dr. Huso is overseeing the pathology. Dr. Dicello's group is establishing the database, compiling the histological results, and analyzing the data. The earliest of four cohorts was completed and submitted for publication. The determinations of the fractions of malignant and benign tumors have now been completed for the 1999 cohorts, with and without Tamoxifen. Plots of these results are presented in this report. Error analyses and internal consistencies are still being examined, so these results are currently confidential and proprietary.

INTRODUCTION

This past year saw new administrative initiatives at both NASA headquarters and NSBRI that will result in major changes in the structure and goals of the Radiation Team. NASA administrators have recommended restructuring the Radiation program within the NSBRI to better support NASA goals. A working group was created, consisting of representatives from NASA Headquarters, NASA Johnson Space Center, NSBRI administration, the Radiation Team and team leaders from other teams. An initial teleconference was held, and this was followed by a meeting of the group at the Brookhaven National Laboratory this past May. Proposed changes include having the members of the Radiation team support radiation studies in other team areas without specific projects assigned to Radiation. The Radiation Team members would also assume major responsibilities in education and mentoring. The NSBRI likewise would supply scientific staff for NASA research in radiation at JSC and possibly other NASA laboratories. The present research projects would be phased out, and future research in radiation or cancer would be supported if it were relevant to the areas of concern for the other teams. Discussions and recommendations are under discussion and consideration. However, in support of the suggested changes, proposals directed toward the Radiation Team were not solicited in the most recent request for proposals issued by NSBRI. As a result, team members have been looking elsewhere if they wish to continue their NASA oriented research, with two members, Drs. Kennedy and Chang, successfully obtaining NASA awards.

Because of changes in the memberships of the External Advisory Council, the Board of Scientific Advisors, and the NSBRI Administration, we reproduce in some detail the background material, including the NASA critical path and risks, and the team structure, described in previous reports.

The risks to human health inherent in space exploration are enumerated in the NASA Critical Path Roadmap, which lists radiation as one of the four Severe Type-I Risks-the most critical type. It follows that the principal goals of the NSBRI Radiation Program are 1) to improve the predictions of risks to human health from space radiations, and 2) to provide effective countermeasures that will significantly reduce these risks. The following risks in the Radiation Effects Discipline Area of NASA Critical Path Roadmap have been identified, and two out of the four are actively being addressed to varying degrees by the Team (NASA risk number in parentheses): Carcinogenesis Caused by Radiation (38), Damage to Central Nervous System from Radiation Exposure (39), Synergistic Effects from Exposure to Radiation, Microgravity and Other Spacecraft Environmental Factors (40), Early or Acute Effects from Radiation Exposure (41), and Radiation Effects on Fertility, Sterility, and Heredity (42).

The radiation risk areas were originally developed for long-term missions, both low-earth orbit or extra planetary, and their relation to the overall space program are shown in the Fig. 1 adapted from NASA's Critical Path Roadmap.

The underlying philosophy of the program's approach is adapted from Dicello (2002) and the NASA report on Modeling Human Risk (1997), i.e., that experimentally determined risks for carcinogenesis and CNS damage in appropriate animal models with corresponding in-vitro measurements can be used to validate theoretical mechanistic relations between animal results and human response. These mechanistic theoretical models, then, can be used to extrapolate known responses of humans to acute exposures of low-LET radiations to expected responses to protracted exposures to protons and HZE particles. When such relations have been established,

Radiation Effects Risk Area

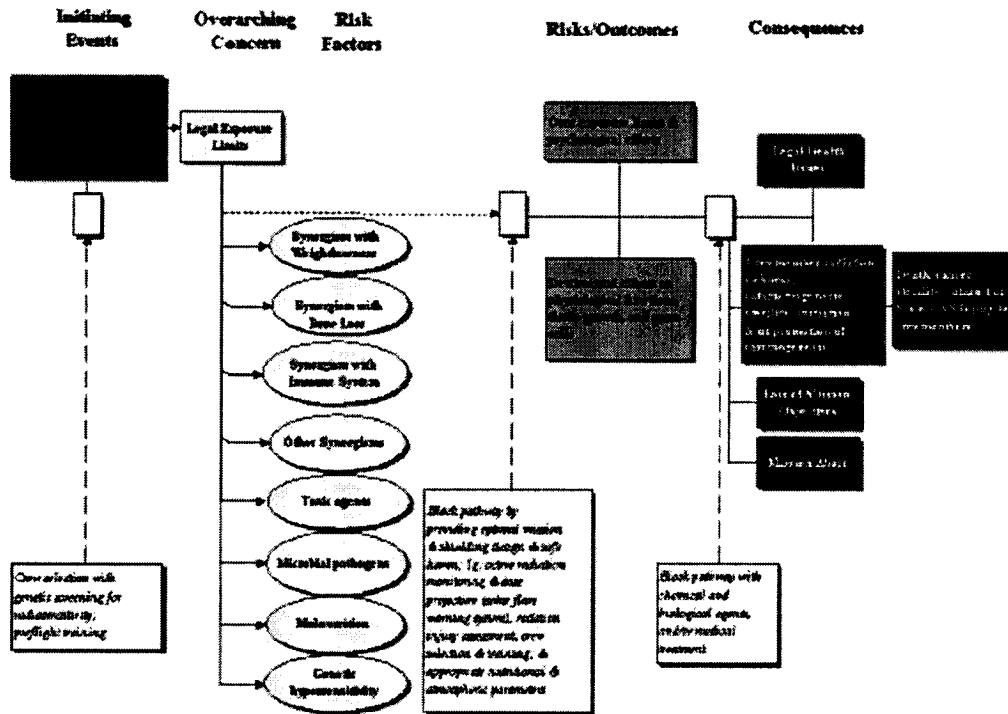
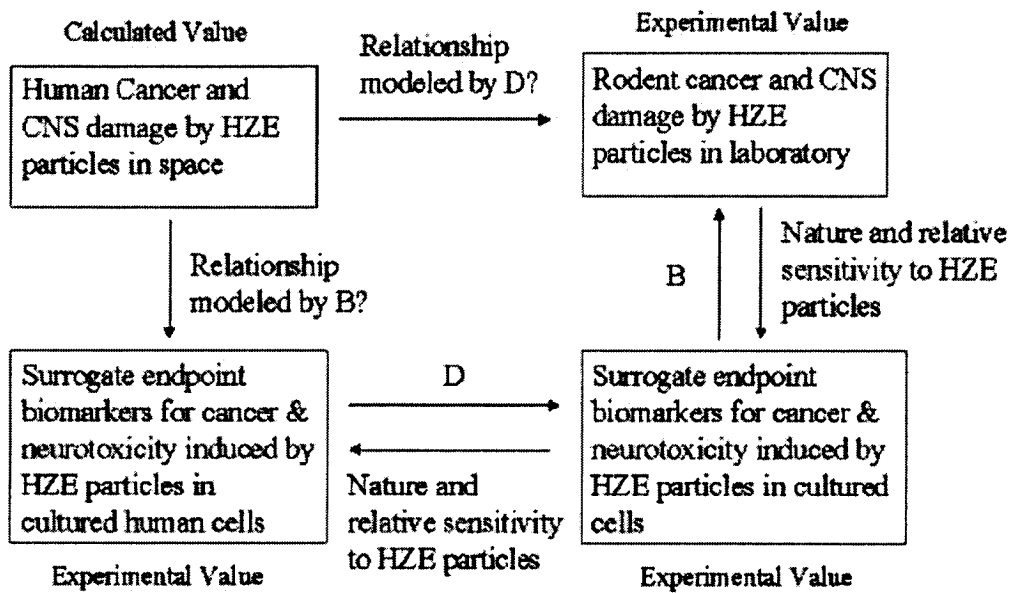


Fig. 1 Roadmap of critical hazards, strategies, and countermeasures

then this same process and these same animal and cell models can be used to determine the potential of pharmaceutical agents, including both chemopreventive drugs and dietary supplements, for reducing risks. This is illustrated schematically in Fig. 2. .



Adapted from Modeling Human Risk: Cell & Molecular Biology in Context, 1997

Fig. V-4. The total number of tumors excised prior to the specified time after irradiation divided by the initial number of animals in that specified

RESEARCH PROGRAM STRUCTURE AND DESIGN

The present Team organization and design, as outlined in Fig. 3, evolved from the original missions of the NSBRI and the Phase-I and Phase-II NSBRI proposals to NASA, as well as the recommendations of the NSBRI External Advisory Council, the NSBRI Board of Scientific Councilors, taking into account the individual projects funded through the peer review system. The overall philosophy is that the Core Project provides in-vivo results for risks of cancer and other diseases and the Chemoprevention Project provides proof of principle and feasibility for risk reduction from low-dose exposures from protons and energetic heavy ions (HZE) using acceptable levels of pharmaceuticals. Tamoxifen was chosen for this benchmark study because it is a drug widely used as a treatment drug for cancer, i.e., therapy rather than prevention, and its effects for x-rays under these circumstances are well known. The Cytogenetics projects, including that of Chang, yield cellular and cytogenetic data for cells irradiated in the animal or in-vitro. The Kennedy et al. investigation of pharmaceutical countermeasures includes a search for nontoxic dietary supplements that will reduce radiation risks. Although the primary risk appears to be cancer, damage to the central nervous system (CNS) may be significant, but the level of risk is poorly known. Dr. Vazquez is examining both the in-vivo effects of proton and HZE exposures and the cellular effects in vitro. Finally, the Core Project assembles all of the data to calculate risks in the animal model. Dr. Dicello has been collaborating with scientists at both JSC and NASA Headquarters, particularly with Dr. Cucinotta's group at JSC, to develop mechanistic theoretical models. This collaboration has resulted in several publications analyzing the experimental data and addressing biological risks already resulting in changes in direction and strategies in reference to different flight scenarios, as well as shielding requirements. Funds for these collaborations with NASA personnel, however, were terminated during the review process.

DESCRIPTION OF CURRENT (FY 2001-2002) PROGRAM

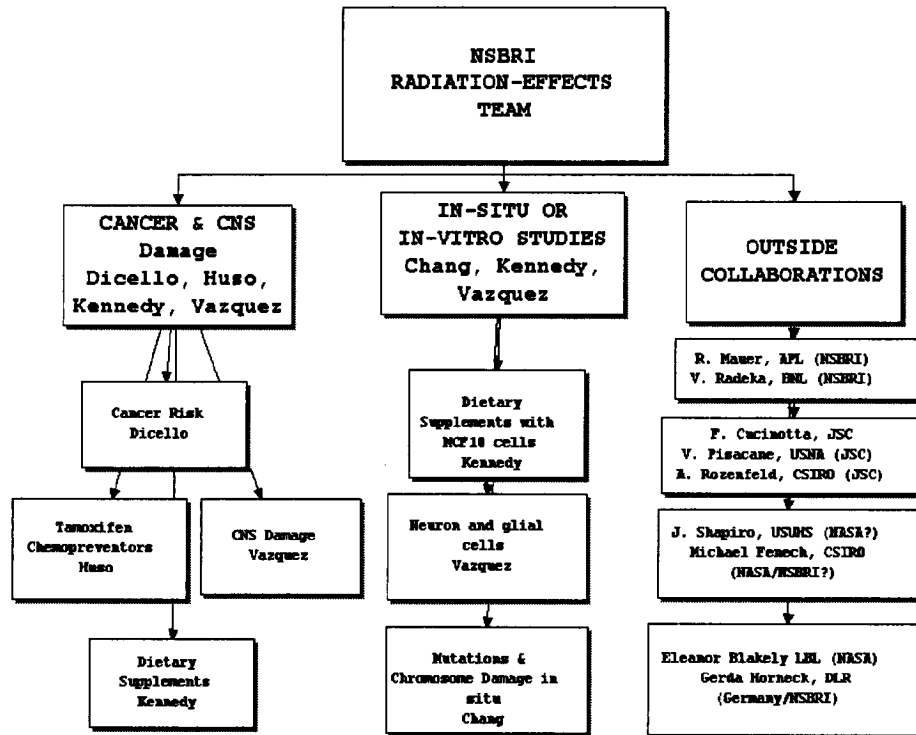


Fig. 3: Present structure of the NSBRI Radiation Team along with active collaborations of members of Radiation with other team members and researchers outside of the NSBRI.

BACKGROUND

Background for the Radiation Effects Team is included here in recognition of the changing composition of the External Advisory Committee.

Almost every review of radiation problems in space has recommended the use of animal studies to quantify the risks to these types of radiation and to pursue promising countermeasures. Until the present series of experiments, there had been only one comprehensive animal study to investigate the effects of ions of high atomic number and high energy, HZEs. Alpen et al. (1993) conducted that experiment with the Berkeley Bevalac, which has been out of commission for almost a decade. Those experiments have provided invaluable data on carcinogenesis in the Harderian gland of a mouse model as a function of linear energy transfer (LET), and they have provided a cornerstone for risk assessments in space during the last decade. No comparable series of experiments had been conducted to evaluate the use of drugs to reduce the risk of cancer from exposures in space.

As a result of scientific reviews, meetings, and discussions during the developmental period of the NSBRI, the Core-Project proposal chose as its animal model the female Sprague-Dawley rat to be irradiated whole-body with HZE's, protons, or photons, in order to evaluate biological

consequences including malignant and benign tumors at all sites, but particularly the breast and the pituitary and other significant diseases. The multiple motives for this choice are presented in detail in the 2000 Final Report for the Core Project; but this choice was ultimately the one recommended by the members of the External Advisory Council. Animal experiments of this type generally were not performed in the past due to the complicated logistics and the substantial expense. Only three facilities in the world, one at Brookhaven National Laboratory in New York state, one in Germany, and one in Japan, produced the necessary accelerator HZE beams. HZE beam time costs millions of dollars a year, far in excess of any funds available from the NSBRI. Until this year, only about 150 hours a year have been available for total space-biology irradiations in the U.S.A. Moreover, no one had ever carried out experiments with energetic charged particles at multiple facilities, including Loma Linda University with its energetic proton synchrotron. (The previous study by Alpen et al. was done at a single site by on-site staff.) The logistics of transporting thousands of animals between multiple facilities in controlled, isolated environments and keeping them alive subsequently for three or more years for cancer studies was at best a challenge. To fully exploit the value of the results, the team offered colleagues in the other projects and other institutions the option to join forces to maximize the production of useful scientific data with the irradiated animals and to provide different information correlated to the same animal species and to humans so that the results could be applied most efficiently to humans in the space environment. Originally, three projects successfully survived the review process initially with two of them joining forces to use the Sprague-Dawley, one studying Tamoxifen (Howard/Huso) as a chemopreventing agent and the other to look at cytogenetics to predict cancer risks (Williams) and to correlate the Sprague-Dawley results to human mammary cells, human lymphocytes, and eight different human colorectal cell lines with varying status of p53 expression. The third project (Sinden) originally proposed to use low-energy helium microbeams to study repeated DNA sequences using techniques that were established at that time only for mouse models. During the intervening time, the principal investigators redirected the project goals to study the more energetic iron beam at BNL and have been developing reporter constructs for the Sprague-Dawley rat. That focused, cooperative effort succeeded in the implementation and execution of one of the most relevant but most difficult series of experiments performed as part of NASA's Life Sciences program in at least a decade. The success of these groups in carrying out these types of experiments has stimulated similar types of radiation studies both in the Radiation Team and in other Teams as well.

At the end of the first three years of the Team program, the investigators had designed, built, and successfully implemented systems to transport and irradiate both animals and cells. Four series of experiments were performed, each examining the consequences of 1-GeV iron ions, 250-MeV protons, and gamma rays from cesium-137 and cobalt-60, as well as sham irradiations. In each case, the animals were irradiated whole body at doses comparable to those expected in space. The animals are cared for and monitored daily and all diseases are medically treated.

We now have statistically significant data for the risk for mammary fibroadenomas and adenocarcinomas and for pituitary-related diseases as functions of particle type, dose, and time, as discussed at length in the project reports. In parallel with the animal experiments, we have been examining cell systems. The principal objectives of these projects were to examine cell survival, cytogenetic damage, and DNA deletions and recombinations, to understand the initial damage and the mechanisms responsible for the initial damage and the subsequent promotion and

progression of the diseases. We are developing theoretical models to simulate the biological alterations and the in-vivo responses. We have used our data and models for preliminary calculations for the risk of carcinogenesis in the animals.

GOALS:

This Team has eight scientific goals for its program: 1) reduction of risk of carcinogenesis caused by radiation; 2) reduction of risk of damage to the central nervous system from radiation exposure; 3) reduction of the risk of synergistic effects from exposure to radiations, microgravity and other spacecraft environmental factors; 4) reduction of the risk of early or acute effects from radiation exposure; 5) reduction of the risk of radiation effects on fertility, sterility, and heredity; and the non risk-based goals of 6) developing methods for assessing level of health risk, prevention of diseases, and appropriate medical care; 7) developing Earth-based applications; and 8) integration of research and analysis.

As mentioned in the Introduction, major changes are taking place in the Radiation Team as it moves away from a research team toward a support resource for the NSBRI and other teams, while, the same time, NASA Headquarters appears to be moving from addressing cancer and countermeasures to stressing basic research, as a result of a recent scientific review of its program. This, of course, raises the question of how to implement the NASA Critical Path Roadmap within the NSBRI format, particularly in reference to determination of cancer risks and pharmaceutical countermeasures. An NSBRI-NASA Steering Group has been established to study these issues and to make recommendations.

RESEARCH PROGRAM ACCOMPLISHMENTS

TEAM ACTIVITIES

The newest principal investigators, Drs. Ann Kennedy and Polly Chang successfully submitted proposals and protocols for beam time at Brookhaven National Laboratory (BNL) and Loma Linda University Medical Center (LLUMC) (protons), and both have only recently completed successful runs at both facilities. Kennedy will return to BNL for the next run in November 2003. Dr. Vazquez also continues CNS studies at BNL and LLUC as well as in Japan. Drs. Dicello and Huso previously completed NSBRI irradiations at BNL and LLUMC, and the animal colonies for studying carcinoma incidence and Tamoxifen intervention are proceeding on schedule. They have not participated in the most recent runs based on an EAC recommendation through Dr. Ron White. One cohort has now produced significant data that have been analyzed and submitted for publication.

A brief description of current research efforts for each investigator is presented, starting with the newer projects, followed by material on collaborations and recent grant and publication activities.

I ANN KENNEDY'S PROJECT ON COUNTERMEASURES FOR SPACE RADIATION BIOLOGICAL EFFECTS:

Introduction:

Exposure to the types of ionizing radiation encountered during space travel is expected to increase the risk for cancer induction in astronauts. The primary types of radiation of particular concern for the astronauts are protons and particles of high atomic number and high energy (HZE particles). The hypothesis to be tested in our NSBRI research program is that control of radiation induced oxidative stress will reduce the risk of cancer development. Our current studies are designed to determine the types of dietary supplement agents or agent combinations that will be the most effective at reducing the level of oxidative stress associated with exposure to ionizing radiation in space. The supplements being evaluated include the following as single agents or as combinations: vitamins C, E, folic acid, glutathione, N-acetyl cysteine, selenium, lipoic acid, niacin, thiamin, Co-enzyme Q10 and the soybean-derived Bowman-Birk inhibitor. The efficacy of the dietary supplement agents is being evaluated in cultured human cells and Sprague-Dawley rats, in which the effects of the dietary supplement agents on the baseline levels of oxidative stress and radiation induced oxidative stress are being determined. For all studies being performed as part of this program, surrogate endpoint biomarkers of carcinogenesis are being monitored, including bio-reduction capacity and oxidative stress in cells and animals. Oxidative stress is measured by the dichlorofluorescein (DCF) fluorescence assay and the protein carbonyl measurement. In animal studies, the level of oxidative stress is measured by determinations of the total antioxidant status (TAS) or the protein carbonyl level in serum or plasma.

The major critical questions the Kennedy program is addressing are as follows:

- A. Risk #38 Carcinogenesis Caused by Radiation; Critical Question Number (from Critical Path Roadmap) 10.03- "Are there chemopreventive or biological agents which would mitigate acute or late effects?"
- B. Risk # 7 Inadequate Nutrition (Malnutrition); Critical Question Number (from Critical Path Roadmap) 5.03 - "What are the nutritional requirements for exploration missions? (e.g., antioxidants, . . .)?"

Recent Findings:

The major findings of the Kennedy laboratory project during the past year are as follows:

- 1) We have developed and optimized a dichlorofluorescein (DCF) fluorometric assay that can reliably detect oxidative stress induced by radiation at doses as low as 1.4 cGy, and can be used to evaluate the effects of various agents on radiation induced oxidative stress in vitro.
- 2) We have compared the efficiency of four types of radiation in inducing oxidative stress in cultured cells and observed that γ -rays, X-rays, protons and HZE particles (1-GeV iron ions) are about equally efficient in inducing oxidative stress in cultured cells.
- 3) We have selected several agents that are effective in preventing radiation induced oxidative stress in vitro. The short list of the selected agents includes ascorbic acid, N-acetyl cysteine, co-enzyme Q10, α -lipoic acid, L-selenomethionine and vitamin E succinate. These agents

lead to significant reductions in the levels of radiation induced oxidative stress in cultured cells, and their effects are consistent and reproducible for all of the different types of radiation sources used to induce oxidative stress in these studies.

- 4) When ascorbic acid, N-acetyl cysteine, co-enzyme Q10, α -lipoic acid, L-selenomethionine (SeM) and vitamin E succinate are utilized together as a combination of agents, the supplement combination is highly effective at reducing the levels of oxidative stress in cultured human breast epithelial cells (MCF10 cells). These agents work synergistically to have a highly significant preventive effect on the levels of oxidative stress.
- 5) The effects of the nutritional supplement combination on malignant transformation were determined in human thyroid epithelial cells (HTori-3 cells) by the soft agar colony formation assay, which measures the capacity of cells to grow in an anchorage-independent manner. The results demonstrate that the frequency of anchorage-independent colonies of HTori-3 cells increased in a dose-dependent manner after exposure to radiation from iron ions or protons. Treatment with the nutritional supplement agent combination prevented the proton and HZE particle radiation induced transformation of HTori-3 cells.
- 6) The effect of radiation on host bio-reduction capacity was determined by measuring the plasma level of TAS in Sprague-Dawley rats irradiated with γ -rays at different doses. The results demonstrated a reduction in TAS in animals exposed to γ -rays at a dose as low as 10 cGy.
- 7) The effects of treatment with the supplement combination described above were evaluated in Sprague-Dawley rats irradiated with 1-GeV or 5-GeV iron ions, protons or γ -rays at a dose of 200 cGy. Sham-irradiated animals were used as controls. The results demonstrated that the TAS decreased in animals fed with the control diet. The control diet (AIN-93G) contained many of the nutritional supplements at levels comparable on a weight basis to the human RNA levels. Supplementation of the diet with the nutritional supplements (at levels comparable to approximately 5 times the RDA levels of the agents) prevented a drop in the serum or plasma TAS after the radiation exposure. These results suggest that diet supplementation was effective in preventing the reduction in TAS in the animals irradiated with all three types of radiation. It was observed that both serum and plasma are suitable for a determination of TAS in the irradiated animals.
- 8) The effects of diet supplementation on radiation induced oxidative stress were evaluated by measuring the plasma protein carbonyl content, an indicator of the protein oxidative damage in Sprague-Dawley rats irradiated with γ -rays at a dose of 200 cGy with sham-irradiated animals used as controls. The results demonstrate a significant increase in the plasma protein carbonyl contents in the irradiated animals fed with the control diet. In contrast, no increase in the plasma protein carbonyl content was observed in the irradiated rats fed with the supplemented diet. These results suggest that there was increased oxidative stress in the irradiated animals and that the radiation induced oxidative stress can be prevented by treatment with the nutritional supplement agent combination.
- 9) The effects of the combination of nutritional supplements on radiation induced cytotoxicity of MCF10 cells were determined by a clonogenic survival assay. The surviving fraction data were plotted against the radiation doses to calculate radiation sensitivity constants according to the multi-target theory using the equation: $S = ne^{-kD}$, where S is the surviving fraction, n

represents the number of targets, $-k$ is the radiation sensitivity constant, and D is the dose of radiation (cGy). The results demonstrate that exposure to HZE particle radiation resulted in a dose-dependent decrease in the clonogenic survival of MCF-10 cells irradiated with 5-GeV iron ions. The radiation sensitivity constants for MCF10 cells irradiated with or without concurrent treatment with the nutritional supplements were 0.0330 and 0.0149, respectively. The doses of radiation required to yield 37% cell survival (known as the D_0 or the D_{37} , which equals $1/-k$) were 30.3 and 67.1 cGy, respectively for the cells irradiated without or with the supplement combination. These results suggest that treatment with the supplement combination protected MCF10 cells from 5 GeV/nucleon iron ion radiation induced cell killing by a factor of 2.21 ($67.1/30.3 = 2.21$).

Implications of Project Findings for Future Research:

The findings that protons and HZE particles are as effective as γ -rays and X-rays in inducing oxidative stress, and that increased oxidative stress was observed in cells exposed to radiation at doses as low as 1.4 cGy, suggest that countermeasures for radiation induced oxidative stress are likely to be essential for preventing radiation induced biological effects in astronauts. The findings that the nutritional supplement agent combination evaluated in our studies can greatly reduce or completely prevent the oxidative stress induced by radiation at doses as high as 200 cGy suggest that diet supplementation with these agents is a feasible and effective approach for the prevention of oxidative stress induced by the types of radiation that astronauts encounter during their travel in space.

Since radiation induced oxidative stress is almost instantaneous and precedes most, if not all, of the other radiation induced short-term and long-term biological effects, countermeasures for radiation induced oxidative stress are likely to be very useful for preventing radiation induced tissue damage and, presumably, cancer development, which are believed to be, at least partially, related to the increased oxidative damage produced by ionizing radiation. Our studies on the biologically relevant endpoints of malignant transformation in vitro and clonogenic survival assays suggest that the nutritional supplement agent combination could have a major suppressive effect on the development of cancer in animals as well as any toxicity resulting from radiation exposures.

Our studies have led to some answers to the critical path roadmap questions listed above, which are: 10.03- "Are there chemopreventive or biological agents which would mitigate acute or late effects?" and 5.03- "What are the nutritional requirements for exploration missions? (e.g., antioxidants, . . . ?)". Our research has suggested that the answer to question 10.03- is yes, there are chemopreventive agents which would mitigate both acute (such as radiation toxicity) and late effects (such as the radiation induced malignant transformation of cells, which leads to the development of cancer as a late effect). From our studies, these chemopreventive agents are ascorbic acid, N-acetyl cysteine, co-enzyme Q10, α -lipoic acid, L-selenomethionine and vitamin E succinate, with a mixture of these agents working synergistically to prevent the acute and late effects expected from exposure to radiation from protons and HZE particles. These same agents, which can represent the types of nutritional requirements discussed in critical path roadmap question 5.03, could be viewed as potentially being essential at some level for exploration missions.

II. POLLY CHANG'S PROJECT ON GENETIC DAMAGE IN TRANSGENIC MICE

Introduction:

We participated in an AGS/BNL run in April 2002 (BNL-8), exposing animals to a range of 1 GeV/amu iron ions from 0.1 – 2 Gy and examined the temporal (up to 16 weeks after exposure) and dose-dependent (0 – 2 Gy) changes in the yield of cytogenetic damage in peripheral blood reticulocytes (MN-RET) and mutations in the reporter transgene (MF) in the spleen at various times after exposure. The following is a brief summary of our results to date.

Dose response of 1 GeV/amu Iron particle radiation induced MN-RET:

We observed significant dose dependent elevation of %MN-RET at ≤ 17 h after whole body irradiation with iron ions. In particular, we demonstrated that these differences were significant at doses as low as 10cGy. Rapid recovery within 72h after exposure was observed in animals exposed to doses ≤ 1 Gy, and delayed up to >120 h for animals exposed to a higher 2 Gy dose. Reticulocytes from both peripheral blood and bone marrow were collected at 8 and 16 wks after treatment and analysis of %MN-RET is now in progress with these late populations of cells. We compared the effectiveness of iron ions in inducing MN-RET to our previous low LET protons results. Preliminary analyses suggest that the relative effectiveness of MN-RET induction in the peripheral blood after iron ion exposure when compared to proton radiation appear to be similar at 48 hr for doses less than 2 Gy.

Analysis of mutations in reporter transgene in spleen tissues:

Preliminary results show that the lacZ MF at 8 wks post iron radiation (BNL8) show a dose-dependence increase at doses from 0.5 – 2 Gy iron particles, but not at 0.1 Gy. We have demonstrated that changes in lacZ MF in tissues are temporally regulated, dose dependent and tissue specific after low LET proton radiation. Although the lacZ MF after 0.1 Gy iron was not significantly different from the controls at this time point, analysis of additional time points and doses are in progress. This information is critical in determining the impact of HZE particle radiation on tissues and is pivotal in the modeling of radiation risks in tissues in humans.

Countermeasure assessments:

Animals were exposed to 2 Gy of protons with or without aluminum shielding. We observed no difference in the yield of MN-RET in peripheral blood at 24 and 48 hrs and lacZ MF in the brain and spleen tissues at 8 weeks after 2 Gy of proton irradiation, with or without aluminum shielding. We recently submitted a manuscript for publication describing these results.

We exposed groups of animals to a single dose of 0.1Gy and 1 Gy iron ions with and without 10-cm of polyethylene shielding, and another group of animals to 1 Gy iron ions but shielded with 15 cm of poly and compared the effect of shielding on the yield of micronucleus in the peripheral blood. We observed no difference between the shielded groups when compared to their unshielded counterparts suggesting the 10- and 15-cm of polyethylene material upstream of the animal was not effective enough as a physical barrier to protect the animal from radiation induced micronucleus induction. These results are consistent with published results (Yang et al, 1998. Adv Space Res 22(12): 1683-1690) and further supports the use of this transgenic mouse model system in the testing of shielding materials in NASA's efforts to develop effective physical countermeasures for radiation protection.

Re: for the period between Feb. to August 2003

Scientific Progress:

Since the last report on 2/24/03, we have continued to collect and analyze samples harvested from the AGS/BNL run in April 2002 (BNL-8). In that experiment, animals were exposed to a range of 1 GeV/amu iron ions from 0.1 – 2 Gy. We are examining the temporal (up to 16 weeks after exposure) and dose-dependent (0 – 2 Gy) changes in the yield of cytogenetic damage in peripheral blood reticulocytes (MN-RET) and mutations in the reporter transgene (MF) in the two tissues, the brain and spleen, at various times after exposure. In addition, we also worked on determining the characteristics of mutants harvested from spleen and brain of proton-exposed animals.

The following is a brief summary of our results to date.

Analysis of mutations in reporter transgene in tissues:

In our previous report, we showed that lacZ MF in the spleen at 8 wks after 1 GeV/n iron radiation show a dose-dependence increase at doses from 0.5 – 2 Gy iron particles, but not at 0.1 Gy. Equal doses of protons appear to be more effective in inducing MF in the spleen than iron particles at this time point.

We have completed our analysis of lacZ MF in brain tissues at 8 weeks after 1 GeV/amu iron ions. Here, we demonstrated that the lacZ MF increased up to >2 fold above control levels after 2 Gy of Iron, suggesting that iron particle induced damage to the CNS tissue persist in this tissue. Moreover, 2 Gy iron particles are more effective in inducing mutations in the transgene than an equidose of protons at this time point.

This information is critical in determining the impact of HZE particle radiation on tissues and is pivotal in the modeling of radiation risks in tissues in humans.

Mutant Spectrum analysis:

We have progressed in the analysis of the Lac Z Mutant Spectrum in brain and spleen tissues at 8 weeks after 1 or 2 Gy proton particle irradiation (Table 1). Mutants were selected at random among the 4 – 6 animals in each treatment group. Isolated plasmid DNA was restriction digested with either Ava I or Pst/Sac or Rsa I restriction enzymes and the RFLP patterns were compared to that of the standard pUR288 plasmid. A minimum of 2 separate digestions, using different enzymes, was used for each mutant to confirm the mutant classifications. Similar RFLP patterns from different mutants selected from the same tissue in the same animal was interpreted as clonal expanded mutants and not considered unique. Of the >100 mutants analyzed, 2 clonal expanded mutants were found in mutants derived from the spleen tissue, and none in the mutants randomly selected from the brain tissues.

Mutant spectrum analysis shows that a predominant number of mutants are ‘no change’ or point mutations for both radiation doses. Whereas the overall percent of point mutations in the spleen tissues remain at about 75% in the controls and after 1 or 2 Gy protons, the percent of mutants in this category increased as the doses increased in the brain mutants. These observations suggest that there are tissue-specific differences in the processing of proton radiation induced damage at

this time point and such mutant characteristics may play a role in determining the level of the residual damage to the genome for long-term risk assessments.

Table 1: Spectrum of lacZ mutants harvested from brain or spleen tissues at 8 weeks after proton exposure.

Tissue	Proton Dose (Gy)	# mutants analyzed	No change	Size Change		Clonal Expansion
				Deletion	Insertion	
Brain	Control	21	16 (76%)	3 (14%)	2 (10%)	
	1	59	50 (85%)	6 (10%)	3 (5%)	
	2	44	40 (91%)	1 (2%0	3 (7%0	
Spleen	Control	43	33 (77%0	8 (19%)	2 (5%)	
	1	25	19 (76%)	4 (16%)	0	2
	2	38	29 (76%)	7 (18%)	2 (18%)	

Future plans:

We received notification that we have 9 hours of approved beam time for the upcoming accelerator run at Brookhaven National Laboratory, and we plan to participate in the NSRL-1 heavy ion beam experiment scheduled in October 2003. We are proposing to expose our transgenic animals to each of the 3 beams (Carbon, Titanium and Iron) available. Preparation for the experiment, including up-breeding of the colonies, coordination of activities including housing, personnel, animal transport, training, and financial arrangements are well underway.

[Analysis of mutation frequency (MF) in the lacZ reporter transgene of mice exposed to acute doses of Gy proton irradiation showed that: spontaneous lacZ MF in the control animals is tissue specific (spleen > brain); MF in the lacZ transgene is responsive to low doses of protons when compared to the unirradiated controls; induction of MF in the **spleen** appear to be dependent on the radiation dose as well as the time post irradiation; persistent enhanced lacZ MF in the **brain** and **spleen** up to 16 weeks post irradiation, suggests proton-induced long-term residual effects in these tissues.

Proton-induced genetic effects are tissue-specific, suggesting that tissue physiology may play important roles in determining the long-term mutagenic potential of low LET particle radiation.]

III. MARCELO VAZQUEZ'S PROJECT ON IN-VITRO DAMAGE TO NEURAL CELL POPULATIONS AND COUNTERMEASURES:

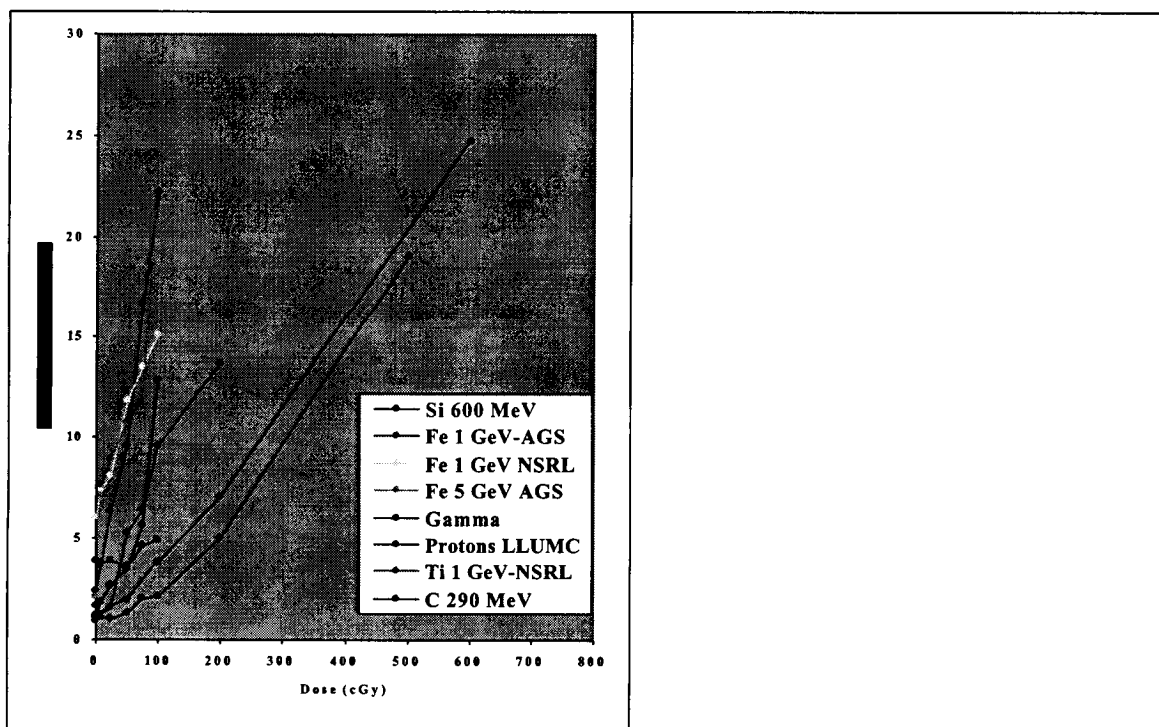
Countermeasure: To protect neural cell populations in vivo using pharmaceuticals, such as neuroprotectants (gangliosides), antioxidants (melatonin) and signal pathways modulators (p53 modulators)

New Research Findings:

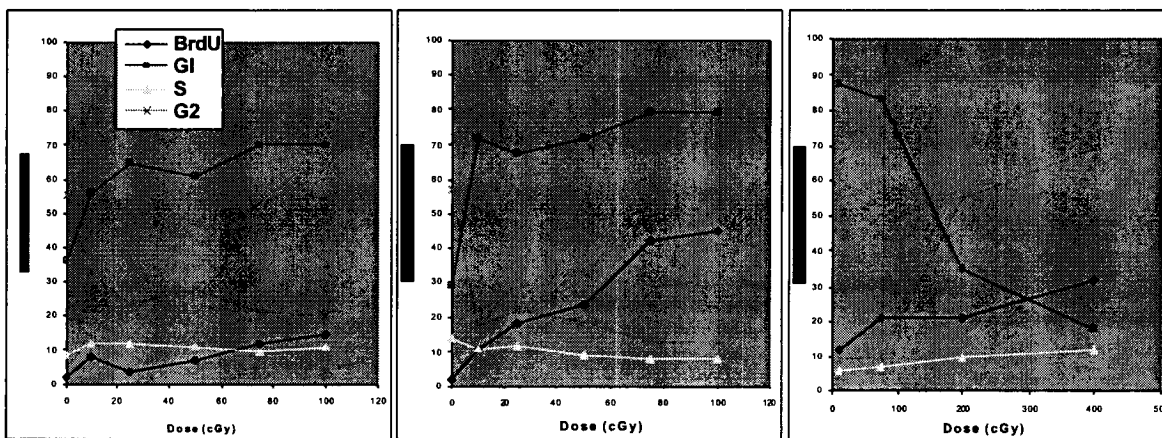
Vazquez: Risk Assessment and Chemoprevention of HZE Induced CNS Damage (In vitro)

Countermeasure: Modulate signaling pathways by pharmacological manipulation (trophic factors, free-radical scavengers, p53 modulators)

During the last fiscal year, we were able to accomplish significant advances in our program. The year 2002-2003 was a period of transition for us due to the commissioning of the new NASA facility, NSRL (NASA Space Radiation Laboratory). During 2002 we ran several experiments at the AGS, and we were the first ever biology users for the new facility getting some important preliminary data. In addition we were part of the cadre of scientists selected for the NSRL commissioning during the summer, performing several experiments using Fe, Ti and C ions. At the present time, we are carrying out several experiments at NSRL as part of the first scientific NSRL run: NSRL-1. Below is the report of the highlights accomplished during the past year.



Effects of low- and high-LET radiation on human neural precursor cells and neurons:In previous runs at AGS and NSRL we were able to collect data using flow cytometry analysis to



The effects of Si ions on cell cycle phase redistribution. NT2 cells were harvested at 48 h post-irradiation and assessed for their cell cycle position. Left panel results at 2 hr, middle panel results at 4 hr and right panel shows results at 24 hr. Individual curves are labeled with respect the cell cycle phase they represent and also a curve representing DNA damage are also included. These results indicate a trend to G1 arrest with time and dose that reverse at 24 hr, at the same time DNA damage level increases as a function of time and dose

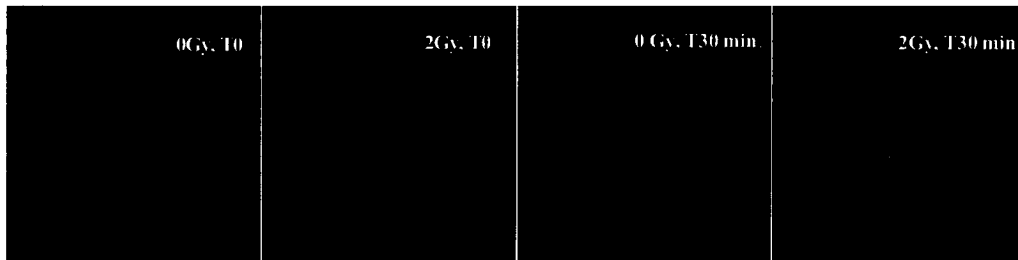
measure the induction of apoptosis in human neural precursor cell NT-2 exposed to low fluences of heavy ions, (Fe, Si, Ti, and C) protons and gamma rays. Cells that stain positively for ANNEXIN V (phosphatidylserine) and negatively for propidium iodine are considered as cells in early stage apoptosis, while those that stain positively for both indicators are either in late stage apoptosis or they are undergoing necrotic cell death. The analysis of the data gathered from several AGS and NSRL runs shows a strong dose- and time-dependent induction of apoptosis in NT-2 cells with the peak of apoptosis appearing at 48 hours post-irradiation. It was determined that Fe and Si ion exposure were more effective to induce than protons and gamma rays, suggesting high RBE values for apoptosis in exposed NT2 cells at 48 hr post-irradiation

Correlation between Fe-induced apoptosis (DNA damage) and changes in cell cycle:

During BNL-9 and NSRL-0 we were able to expose NT-2 to Si and Fe ions to detect changes in cell cycle distribution and correlated DNA damage. Results obtained from our Si run are shown below. Methods and techniques are described below in experimental design. These results are the product of our active collaboration with Dr. Lora Green at Loma Linda University Medical Center. Iron samples are still being processed, and additional samples will be taken during NSRL-2.

γ -H2AX foci formation to monitor DSBs in NT2 cells

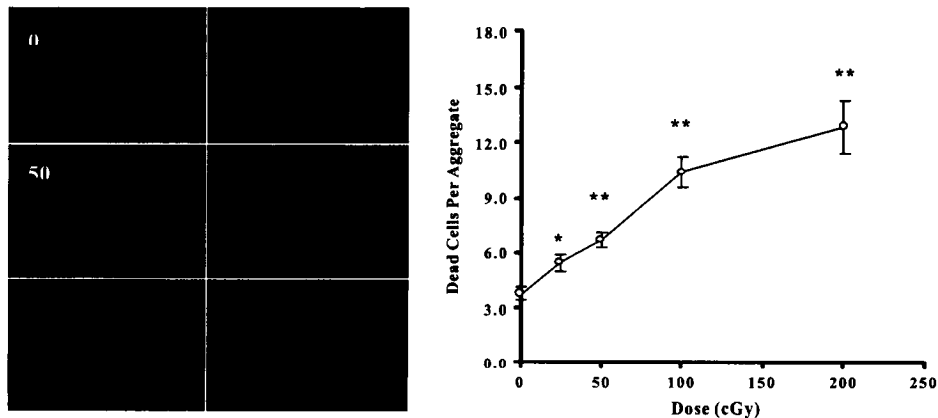
The appearance of nuclear γ -H2AX foci constitutes a bona fide marker of the cellular stress response to DNA damage. Rothkamm and Löbrich, (2003) demonstrated that foci of γ -H2AX (a phosphorylated histone), detected by immunofluorescence, are quantitatively the same as DSBs and are capable of quantified repair of individual DSBs. This finding allows the study of DSB repair after radiation doses as low as 1 mGy, an improvement by several orders of magnitude. Phosphorylation of histone H2AX (γ -H2AX) is among the earliest ATM-dependent responses to DSBs (Burma et al., 2001). Recently, it has been demonstrated that loss of H2AX in mice



γ -H2AX foci formation in NT2 cells fixed 0 and 30 minutes h after γ -irradiation (2 Gy).

results in genomic instability and defects in concentrating various DNA repair factors into radiation-induced nuclear foci (Celeste et al., 2002). We have initiated preliminary studies on the use of γ -H2AX to monitor DSB in human neural precursor cells (NT2) induced by ionizing radiation. These studies examined the ability to detect foci formation after exposure to gamma radiation as a function of time. Samples were processed for immunolocalization of γ -H2AX foci using an anti-phospho-H2AX antibody (Trevigen, Gaithersburg, MD). Results suggest that γ -H2AX foci formation disruptions to the phosphodiester backbone of DNA signal this protein complex to initiate recombinational repair. Preliminary data indicate clearly the capacity to detect foci formation following ionizing radiation in NT2 cells.

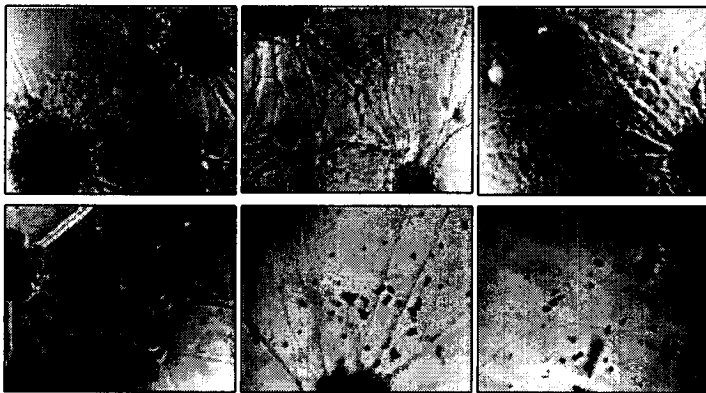
Neurotoxic effect of 1 GeV/n Fe ions on neural cells



Dose-dependent increase of apoptosis in neuron aggregates exposed to Fe ions. hNT cells were exposed to graded doses of 1 GeV/n Fe ions and samples were taken at 30 days in culture after exposure. **Left Panel:** the number of apoptotic cells per aggregate was measured using Live/Death kit (Molecular Probes) and fluorescent microscopy at 30 days after exposure. Green images show healthy neurons. Same aggregate visualized with a different filter show apoptotic nuclei stained in red. **Right Panel:** dose-response curve for the number of apoptotic cells per aggregates in cultures exposed to iron ions. Data shows a dose dependent increase of neuron damage with doses as low as 25 cGy at 30 days in vitro. Similar responses were obtained at earlier time points.

Gene expression studies/p53 pathway: p53 expression in NT-2 and hNT cells exposed to Fe ions

Conventional western blot techniques were employed to monitor p53 gene expression. A subset of NT2 and hNT cell samples was lysed at different time points for the determination of p53 expression using monoclonal antibodies against human p53. We observed that p53 is over-expressed as a function of dose and time post-exposure in both cell lines. Doses as low as 0.25 Gy were able to up-regulate p53 and as early as 8 hours post-exposure. Doses of 0.75 Gy were able to up-regulate p53 as early as 12 hours post-exposure. Similar results were obtained when NT-2 cells were exposed to gamma or proton exposures, although higher doses were required to obtain a similar degree of expression. These results confirm that the p53 gene is involved in the stress pathway induced by low- and high-LET radiation exposures.



Effects of iron ions on neuron aggregates neurite outgrowth at 30 days in culture. Top panel shows control cultures with extensive neurite networks. Bottom panels shows marked decrease of neurite mass and cellular debris after 200 cGy of Fe particles.

IV. MARCELO VAZQUEZ'S PROJECT ON IN-VIVO CNS DAMAGE AND COUNTERMEASURES:

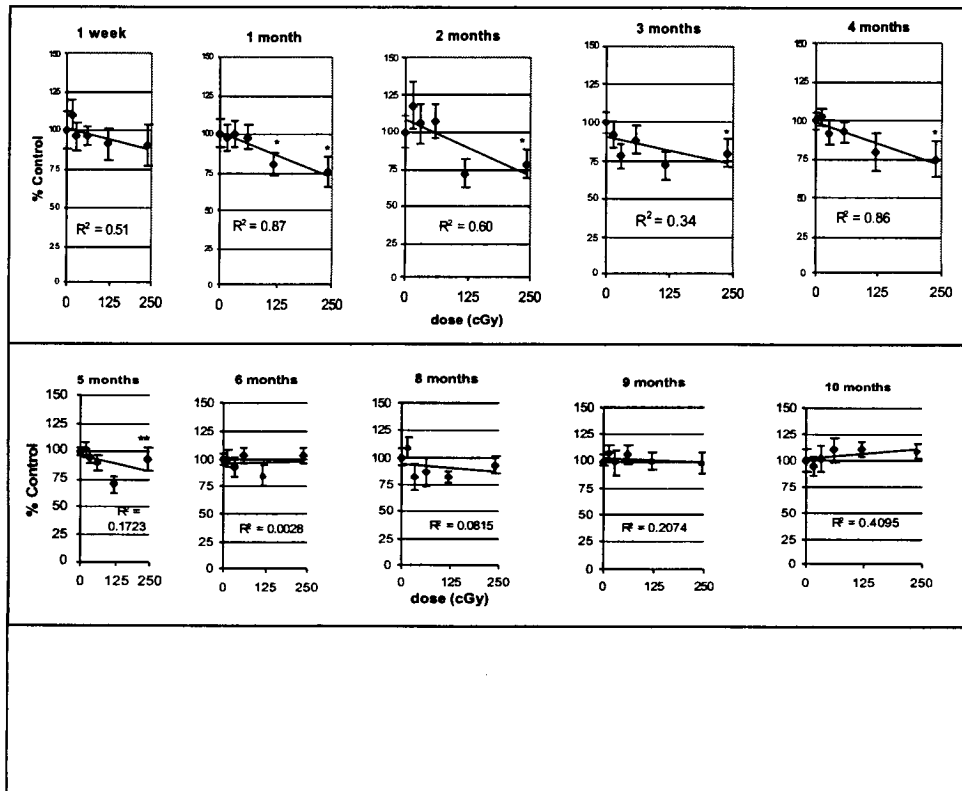
Countermeasure: To protect neural cell populations in vivo using pharmaceuticals, such as neuroprotectants (gangliosides), antioxidants (melatonin) and signal pathways modulators (p53 modulators).

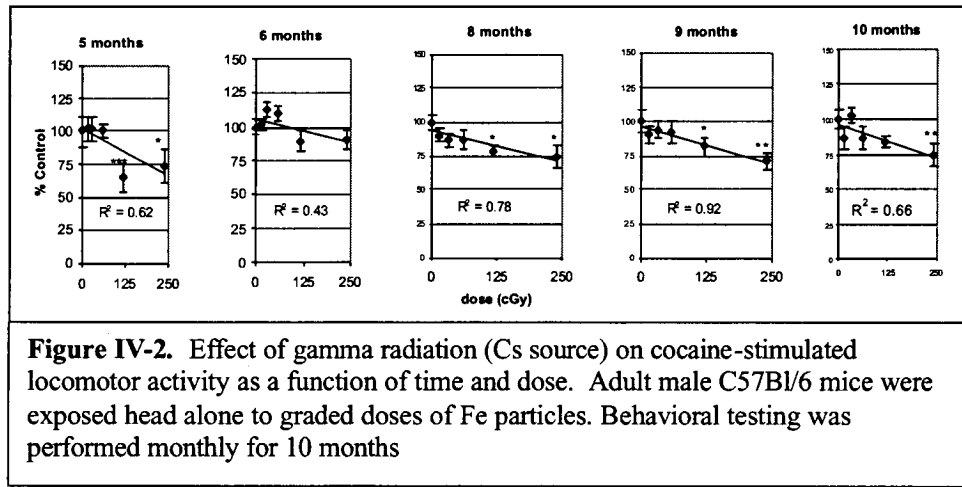
Goals:

- 1) Characterize the behavioral consequences after exposure to low-fluences of heavy ions and protons on C57BL/6 mice. The main behavioral endpoints to be used in these studies are locomotor activity to evaluate the integrity of striatal dopaminergic pathways, and spatial reference memory to probe hippocampal cholinergic pathways.
- 2) Characterize the neurochemical and structural changes induced by heavy ions and protons.
- 3) To develop countermeasures to protect neural cell populations exposed to low fluences of heavy ions and protons.

New Research Findings:

Iron particle irradiation caused dose related reductions in locomotor activity stimulated by cocaine, as evidenced by the group data presented here. The impairments after HZE radiation appeared to be persistent (Figure 1). Irradiation using a Cs source also led to alterations in cocaine-stimulated locomotion at early times, but, unlike the situation for HZE radiation, these disappeared at later times. (Figure 2). These studies were very recently terminated and data analysis is not yet complete. For example, spontaneous activity was also monitored, and it is possible that comparison of stimulated and spontaneous locomotion for each animal may expose larger changes.





Most of the mice were sacrificed and their brains stored for histology and neurochemistry. Ex vivo determination of dopamine transporter status in striata of some of the mice indicated no large decrease in this marker of pre-synaptic dopamine terminals, supporting an earlier pilot study in rats (Gatley et al., 1999).

During BNL-9, we evaluated the effects of HZE radiation in mice with pre-existing neurological damage induced by methamphetamine. The rationale for this study is that behavioral radiotoxicity may become exposed in animals, which have been already compromised, by

administration of a neurotoxin. Recent PET human studies have found decreases in DA transporters and D2 receptors induced by methamphetamine that are correlated with decrements in motor and memory tasks. Mice were pretreated with methamphetamine (METH, 10 mg/Kg 4 times subcutaneously in a single day) before heavy ion irradiation. Our initial working hypothesis suggested that an additional loss of these cells due to HZE radiation might induce behavioral deficits at lower radiation doses than in animals not treated with methamphetamine. Results gathered during BNL-9 (Figure 3) failed to demonstrate our initial hypothesis. In fact, Fe-induced decrements in locomotor activity were not only prevented but also increased in methamphetamine pre-treated animals at 12 weeks after irradiation. We believe that these results do not indicate that METH has a neuroprotective effect on Fe-exposed animals. Rather, this increase could be a manifestation of compensatory mechanisms or neurotoxicity. Further studies are needed to confirm these results and expand the studies with a more robust neurochemical analysis.

Dose	HZE	Gamma
0	2.44±0.41 (12)	2.44±0.41
15	2.33±0.40 (4)	2.30±0.39 (4)
30	2.28±0.53 (4)	2.46±0.17 (3)
60	2.40±0.56 (4)	2.64±0.26 (4)
120	2.48±0.35 (8)	2.30±0.37 (6)
240	2.06±0.37 (6)	2.46±0.34 (7)

Dopamine Transporter Binding. Values are the mean±s.d. of the ratio of radioactivity concentration in striatum and cerebellum 30 min after injecting [³H]WIN 35,428.

During BNL-9, mice were exposed to Fe particles to study the effects of heavy ion exposure on learning and memory using the Morris water maze paradigm. Results are shown on Figure 4. 1

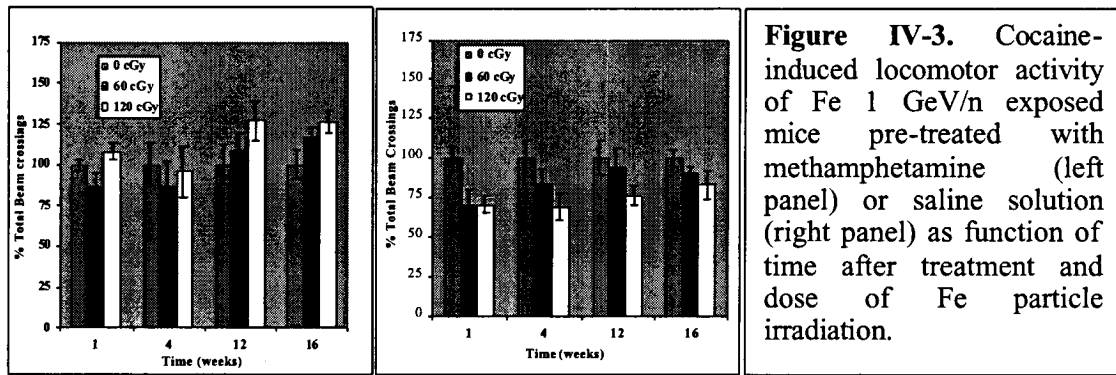


Figure IV-3. Cocaine-induced locomotor activity of Fe 1 GeV/n exposed mice pre-treated with methamphetamine (left panel) or saline solution (right panel) as function of time after treatment and dose of Fe particle irradiation.

GeV/n Fe ion exposure induced a dose- and time-dependent increase in latency times, reaching a maximum level at 3 months post exposure. Effects persisted up to six-month post-irradiation expressing long-lasting effects on learning and memory processes. Neurochemical analysis of collected brains is under study to determine morphological and neurochemical changes at the hippocampal region induced by Fe particle exposures.

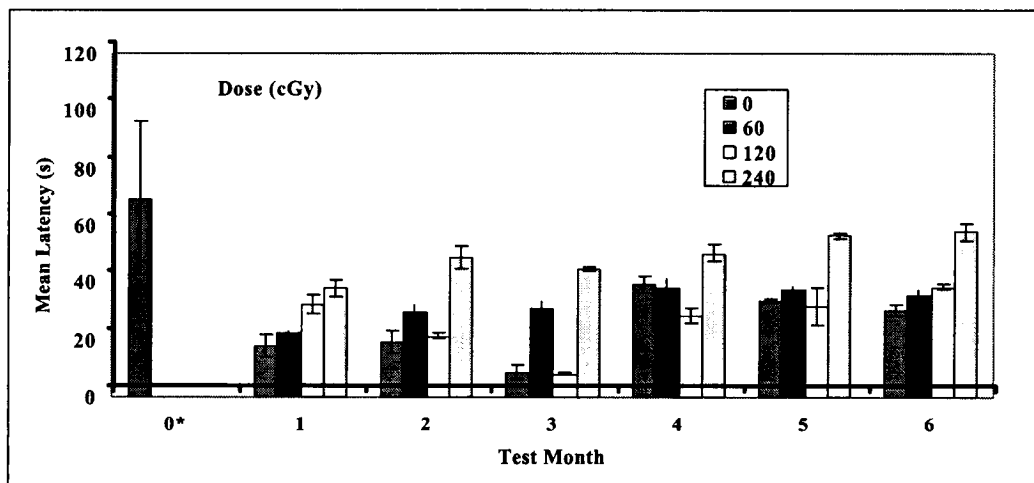


Figure IV-4. Effects of 1 GeV/n Fe particles on spatial performance and memory using the Morris water maze as a function of dose and time post-exposure. C57Bl/6 mice were pre-trained before Fe exposure (month 0*) to learn the task and tested up to six month after radiation exposure.

V. JOHN DICELLO'S PROJECT ON –IN-VIVO MAMMARY CARCINOMAS:

Countermeasure: A precise knowledge of the risks for different types of cancers as well as the lifetime risk for cancer, along with the genetic and epigenetic factors responsible for variations in the risks among individuals will lead to optimal spacecraft designs and mission scenarios. Further, comparable data for potential chemopreventive pharmaceuticals can result in reduced risks for the same mission scenarios. With the Tamoxifen data of Huso's group, our analysis is showing that pharmaceutical intervention in the promotion or progression phases of cancer, rather than in the initiation stage, can reduce the risk of cancer. This suggests the potential for treatment for prevention at relatively long intervals following exposure rather than before or immediately after.

Recent Research Findings:

Part of the following material was included earlier this fiscal year in a report to the External Advisory Council. Animals in all four cohorts irradiated with iron ions, protons, and photons as well as sham irradiated are now deceased. The histology of mammary tissues for the first cohort was completed and results were submitted for publication previously. The paper was accepted subject to extensive revision. Sample preparations for the remaining animals are being carried out, and completed samples are being examined and classified.

For our animals, the average number of carcinomas was one carcinoma per rat with twelve glands at risk. After irradiation, the risk generally increases with dose but continues to remain relatively low. With whole-body irradiation, this average number increases to between three and four, roughly independent of the radiation type. Mammary cancer is a disease of older animals with tumors appearing in the first hundred to two hundred days, and then a relatively constant increase in risk. Qualitatively, the shape of the risk curve as a function of time appears to be roughly independent of particle type. Likewise, the relationship between doses and particle types appears to change multiplicatively in absolute risk rather than shifting to earlier onset of the disease.

The first cohort was not originally intended to be a comprehensive study but rather was meant to serve as a preliminary survey for defining the subsequent protocols. However, that series has produced a significant amount of data. This first cohort did not show a significant difference in the incidence of cancer at the lowest doses among the three types of radiations when the animals are considered in toto. However, an analysis of subgroups indicates hormone dependent differences in tumor induction that are observable and statistically significant even at the lowest doses. An important inference for subsequent experiments is that differences in carcinogenesis of approximately 30% or more were able to be resolved. Moreover, there were histologic and genetic differences observed among the tumors induced by the radiations in comparison with those occurring naturally, and described in an article in press.

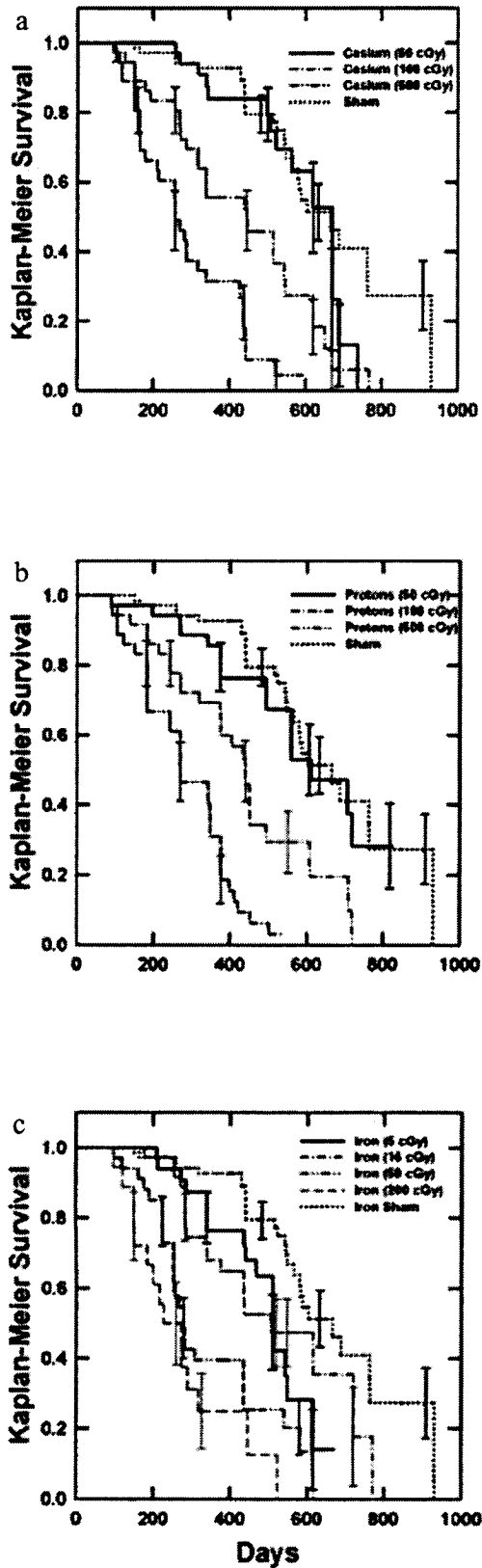


Fig. V-1a-c

Recent Analysis:

For the 1998 cohort, Kaplan-Meier curves for survival of rats with carcinomas as a function of time are presented in Figs. 1a through 1c for animals exposed to 5, 16, and 50 cGy of 1-GeV/nucleon iron ions; 50, 160, and 500 cGy of 250-MeV protons; and 50, 160, and 500 cGy of cesium-137 gamma rays (BNL). Error bars are presented for selected data points rather than for points for clarity. Error bars in these and subsequent plots refer to statistical variations only, assuming random and independent occurrences. The average fraction of lifetime mammary carcinomas as a function dose for animals exposed in 1999 is plotted in Fig. 2, and for animals subsequently receiving Tamoxifen (D. Huso's project) in Fig. 3. The lifetime incidence of mammary cancer in rats is dependent upon the particular line, diet and other care factors as well as histologic criteria, and it can vary widely. For our animals, the average number of carcinomas was one carcinoma per rat with twelve glands at risk. After irradiation, the risk generally increases with dose but continues to remain relatively low. With whole-body irradiation, this average number increases to between three and four, independent of the radiation type. The total number of tumors as a function of time divided by the total number of animals initially irradiated in that group is presented in Fig. 4. The integral response functions for excess cumulative incidence of rats with mammary carcinomas in excess of the natural incidence as a function of dose for gamma rays, protons, and iron ions are shown in Fig. 5. The lowest data point for each of the three curves does not show a significant difference in comparison with the shams. All other data points have p values less than 0.05.

Relative Biological Effectiveness:

Although absolute risk is the major endpoint for the present application, we have examined the relative biological effectiveness (RBEs) for our results. Although the applicability of the concept of an RBE for the present data is called into question by the relative shape of the dose response curves, such an analysis does allow us to compare our results more directly with previous studies. This analysis was presented to the NASA

community at the NASA Workshop this past January.

Within the uncertainties of the data, we have determined values of RBEs at specified doses, but the values vary depending upon the doses chosen. Frequently, theoretical models are used to extract an RBE by extrapolating below the lowest doses, but there is no single calculation or theoretical model that can be used to unequivocally produce an RBE. We are carrying out extensive analyses of these data to extract relative risks and, if possible, the RBEs using different approaches. We have provided our data to scientists at the NASA Johnson Space Center who are analyzing the data with other approaches as well. Having noted the caveats about RBEs, it is nevertheless irresistible to compare with the previous results reported by Fry et al. (1985) and Alpen et al. (1993, 1994) for tumorigenesis in the Harderian gland of the mouse and by Burns et al. (1993, 2001) for skin cancer in the mouse.

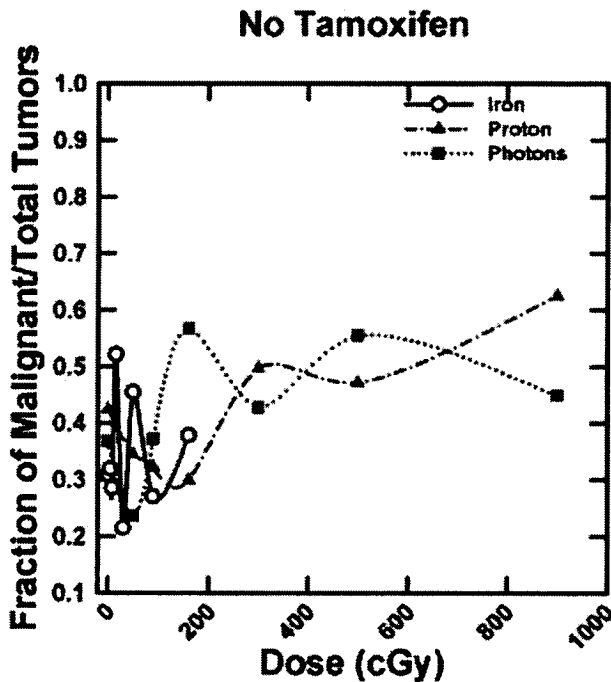


Figure V-2

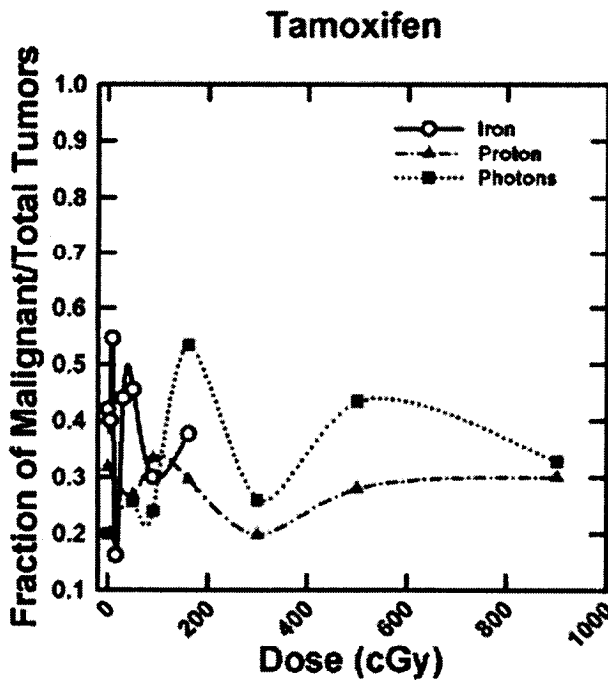


Figure V-3

If we calculate directly from the spline fits to the data shown in Fig. 5, we obtain RBEs for excess incidence of mammary carcinomas of approximately 16 ± 4 at 5 cGy, and 6 ± 2 at 16 cGy. Shellabarger et al. (1980), for comparison, obtained an RBE of approximately 12 at a dose of 6.4 cGy of 430 keV neutrons (with the RBE rising to about 200 at a dose of 0.1 cGy). If we use the approach of Alpen et al. (1994) by taking the ratio of the initial slopes, although again it should be stressed that a same linear-quadratic relation is not the best fit, we obtain an RBE in the range between 7 and 13. This value for our endpoint, mammary carcinomas in the Sprague-Dawley rat, is lower than the value of (39 ± 12) obtained by Alpen et al. (1993) for tumor prevalence in the Harderian gland obtained for iron ions at the lower energy of 600 MeV/nucleon,

but the uncertainties were comparable to the observed difference. (Although our energy is somewhat higher (lower linear energy transfer, LET), in a companion study, we degraded the average energy of the iron beam to about 600 MeV/nucleon and saw no significant change in the response). One additional study with which to compare is a project still underway, in which Burns (1993) irradiated male Sprague-Dawley rats with 640-MeV/nucleon argon ions and compared the resulting yield of skin cancer, primarily fibromas, with that from 1.8-MeV electrons. Although they prudently did not provide RBEs in their papers, and the slope for the electrons continues to decrease faster than linearly, a lower value in the neighborhood of 5 would appear to be reasonable in the region where there are data. In the latest paper, the same group reports that single doses of 1-GeV/nucleon iron ions are two or three fold more effective than argon in producing tumors.

Implications of Project Findings for Future Research:

Our results show the potential for reducing the risks of high-LET exposures in space through chemopreventive intervention in the promotion and progression stages of the diseases rather prior to exposure or immediately after. In addition to examining mammary cancers, we have examined our animals and quantified pituitary tumors, hematologic malignancies, and intestinal tumors. The objective of this research has been to examine

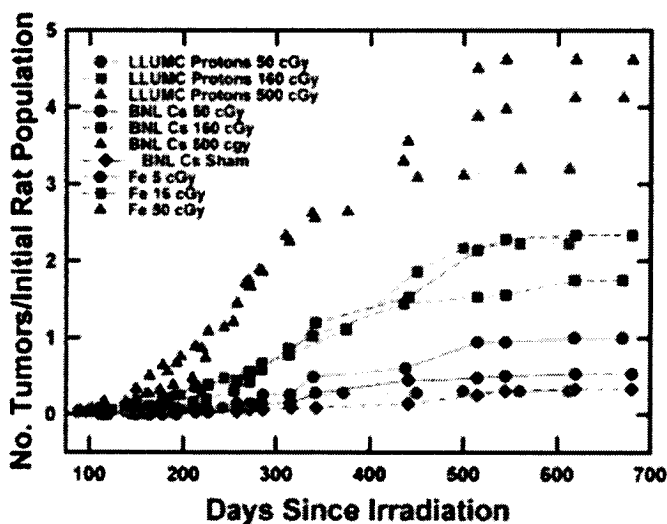


Fig. V-4. The total number of tumors excised prior to the specified time after irradiation divided by the initial number of animals in that specified group. Only a representative sampling of the total number of groups is presented for purposes of clarity.

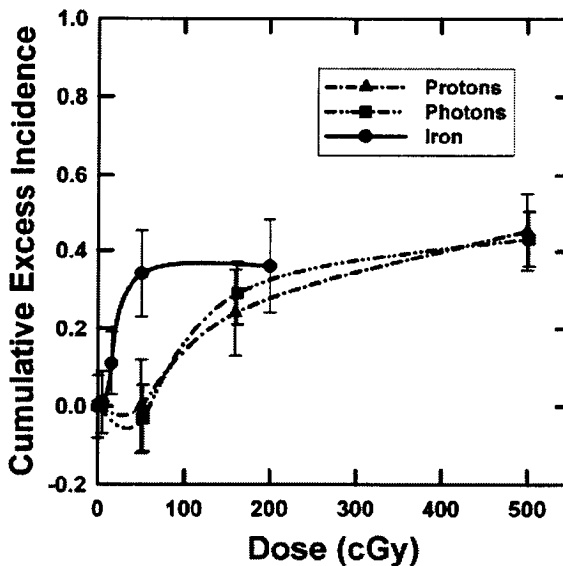


Fig. V-5: Lifetime excess incidence of mammary carcinomas as a function of dose for gamma rays, 250-MeV protons, and 1-GeV iron ions.

carcinogenesis and major processes contributing to increased risks for cancers. The approach will take advantage of recent advances in stem cell and molecular biology by our collaborators and us in projects outside of the NSBRI. Specifically, our group has developed newer transgenic and transduction models to isolate, purify, and track the earliest pluripotent stem cells available. Tracking can continue to a somewhat later phase of stem cells, and still later phases of bone marrow and hemo progenitor cells through to clinically observable carcinogenesis. This novel method will offer the opportunity to identify tagged stem cells and their progeny in hematopoietic or other tissues during any stage of development in order to characterize clinical and molecular factors and changes in these cells relative to the other populations or controls. Because the loss of bone mass during extended missions is one of the most critical risks observed for personnel in Space, we are proposing to particularly examine both the hemoprogenitor and osteoprogenitor cell populations in the bone marrow subjected to radiation and/or simulated microgravity using hindlimb suspension. Because this is such a novel approach, the foregoing discussion in this section is confidential and proprietary.

We began downsizing in anticipation of the completion of the project by the end of December. We have requested a no-cost extension to permit us to complete the project with a reduced work force over a longer period of time. This would also serve the purpose of allowing the group to remain active in the NSBRI until NSBRI and NASA officials determine the new structure and goals for radiation research and the Radiation Team.

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DAVID HUSO'S PROJECT ON CHEMOPREVENTION AND RADIATION-INDUCED NEOPLASMS:

Countermeasure:

The use of Tamoxifen as a model for pharmaceutical intervention in the promotion and progression stages of carcinogenesis to reduce risk after exposure.

New Research Findings:

The three cohorts of Sprague-Dawley rats that included arms receiving lifetime Tamoxifen after irradiation are now completed. Preliminary analyses show that Tamoxifen markedly reduces the incidence (reduces slope) and prolongs the latency (shift to the right) of mammary carcinomas in animals following iron ion radiation exposure. The Tamoxifen chemoprevention will be similarly effective following exposure to photons. The preliminary analyses showed a somewhat reduced effect with protons, but it is not known yet whether that difference persisted throughout the life of the animals.

Our study showed that long-term Tamoxifen chemoprevention and aging in the Sprague Dawley rat model appears to have complex direct and indirect effects on reproductive tract tissues that are under the influence of the ovarian-pituitary axis hormones. The majority of the 15-month-old control rats 62.5% (5/8) were cycling regularly and 25% (2/8) had an irregular cycle. This was considered a high level of cycle activity considering their age. None of the 15-month-old rats in the treated (irradiated, Tamoxifen, or both) group were cycling regularly, but 9/21 had an irregular cycle. This suggests that Tamoxifen and radiation may result in the onset of senescence of the reproductive cycles in rats while directly altering cervicovaginal morphology.

We have chosen Tamoxifen chemoprevention to provide initial "proof of principle" studies on the chemoprevention of radiation-induced cancer. Our focus has been on initiation of chemoprevention following (30 days post exposure) known radiation exposure. Over the past several years, Tamoxifen has become the most widely prescribed anticancer drug in the world, as it is widely prescribed for breast cancer. Recently, it has also become one of a handful of chemopreventive compounds that have been clearly proven to be effective in the chemoprevention of human cancer in large-scale clinical trials. However, its safety and efficacy as a countermeasure against the potential carcinogenic effects of radiation exposure in space is entirely unknown. Tamoxifen is the prototype for a group of chemopreventives that act by modulating the estrogen pathway and interfering with the early growth of cells that could become cancerous.

Interestingly, following radiation exposure carcinomas that develop at times display features of both mesenchymal and epithelial neoplasms. Although some of the cancers appear to be spindle cell tumors of mesenchymal origin, recent immunohistochemical analysis in our laboratory has shown that these tumors still express varying levels of keratin, a marker of epithelial differentiation. This suggests that they may arise from primitive stem cells that can differentiate along different pathways. We are currently investigating global gene expression differences that may further explain the radiation target cell mechanisms that result in these morphologically distinct neoplasms.

Our studies also show that Tamoxifen markedly reduces the incidence (reduces slope) and prolongs the latency of mammary carcinomas following exposure to ionizing radiation. It appears that Tamoxifen chemoprevention is similarly effective following exposure to iron ions and protons. Our studies suggest that chemopreventive administration beginning one month following radiation exposure can prevent a significant proportion of radiation-induced cancers by acting during the promotion and progression stages of carcinogenesis. This is important because it provides the option of doing a risk/benefit analysis for individuals based on actual levels of radiation exposure during space travel before prescribing chemopreventives. This is in contrast to agents that must be present during the time of radiation exposure in order to be effective.

Similar positive trends are emerging concerning Tamoxifen impact on improving survival following irradiation. However, Tamoxifen chemoprevention failures do occur, particularly late in life. We are investigating these late occurring cancers and possible reasons for Tamoxifen chemoprevention failures later in life. These mechanistic insights may provide the basis for combining new approaches with strategies aimed at altering the estrogen pathway. Optimum chemoprevention may benefit from a combinatorial approach using chemopreventive strategies that act on different pathways in radiation-induced carcinogenesis particularly to avoid cancers that occur later in life.

One of the potential reasons for Tamoxifen chemoprevention failures that we have been investigating is the heterogeneity of the effects of Tamoxifen on the ovary and the ovarian cycle. This becomes particularly obvious later in life. Tamoxifen did decrease early tumors and improve longevity following irradiation. We found that there was generally granulosa cell hyperplasia in the ovarian tissue of individuals receiving Tamoxifen chemoprevention. Most individuals reached reproductive senescence by 15 months of age and ceased cycling. However, some individuals receiving Tamoxifen chemoprevention continued with regular or irregular ovarian cycle activity well into old age (15-25 months of age). These individuals would normally have already undergone reproductive senescence or would have been considered postmenopausal. This age group appears to also be the group most likely to experience sporadic Tamoxifen chemoprevention failures in terms of mammary carcinomas. We conclude that heterogeneity in the response of the ovarian cycle and hence hormones of the pituitary-ovarian axis, could explain a portion of the Tamoxifen chemoprevention failures that occur later in life.

Table 1. Ovarian cycle activity later in life during Tamoxifen chemoprevention

	Radiation ^a				Tamoxifen & Radiation ^b				Tamoxifen ^c				Control ^d			
	C	N	Ir	ETC	C	N	Ir	ETC	C	N	Ir	ETC	C	N	Ir	ETC
15 month	0/5	2/5	3/5	0	0/5	2/5	3/5	1	0/11	8/11	3/11	1	5/8	1/8	2/8	0
20 month	0/5	4/5	1/5	0	0/5	4/5	1/5	3	1/10	6/10	3/10	0	0/8	8/8	0/8	0
25 month	0/5	5/5	0/5	0	0/4	4/4	0/4	0	1/11	9/11	1/11	1	1/7	5/7	1/7	0
Total:	0/15	11/15	4/15	0/15	0/14	10/14	4/14	4/14	2/32	23/32	7/32	2/32	6/23	14/23	3/23	0/23

C=cycling N=not cycling Ir=irregular cycle ETC=endometrial-type cells

Fifteen-Month-Old Rats:

- ^a *N* represents diestrus in two rats for four consecutive days.
Ir represents two days estrus with two days metestrus in one rat, and three days estrus with one day diestrus in one rat
- ^b *N* represents diestrus in two rats for four consecutive days.
Ir represents two days estrus with two days diestrus in two rats, and one-day diestrus with three days estrus in one rat.
- ^c *N* represents metestrus in three rats, late metestrus/early diestrus (M/D) in one rat, two days metestrus and two days diestrus in four rats.
Ir represents estrus in three rats for four days.
- ^d *N* represents metestrus in one rat for four days.
Ir represents two days metestrus, one-day estrus, and one-day metestrus in one rat, and one-day estrus with three days metestrus in one rat.

Twenty-Month-Old Rats:

- ^a *N* represents M/D in two rats, diestrus in one rat, and metestrus in one rat for four consecutive days
Ir represents one-day proestrus plus three days estrus in one rat
- ^b *N* represents metestrus in two rats, diestrus in one rat for four consecutive days, and two days metestrus and two days diestrus in one rat
Ir represents estrus in one rat for four consecutive days.
- ^c *N* represents diestrus in five rats, and M/D in one rat for four consecutive days.
Ir represents estrus in one rat for four days, and two days estrus with two days metestrus in one rat
- ^d *N* represents one-day metestrus with three days diestrus in three rats, three days metestrus with one-day diestrus in four rats, and diestrus in one rat for four consecutive days.

Twenty-five-Month-Old Rats:

- ^a *N* represents metestrus in two rats, M/D in one rat for four consecutive days, and two day metestrus plus two day diestrus in one rat.
- ^b *N* represents metestrus in two rats for four days, M/D in one rat for four days, and two days metestrus plus two day diestrus in one rat
- ^c *N* represents diestrus in six rats for four consecutive days, M/D in two rats for four consecutive days, and metestrus in one rat for four consecutive days
Ir represents as follows (*di* + *di* + late estrus + early estrus)
- ^d *N* represents two days metestrus with two days diestrus in one rat, and M/D in four rats for four consecutive days
Ir represents three days diestrus with one-day estrus in one rat.

Table 2. Summary of ovarian histopathology at 15, 20, and 25 months by types of treatment.

	Radiation				Tamoxifen &Radiation				Tamoxifen				Control			
	GH	IA	FO	CL	GH	IA	FO	CL	GH	IA	FO	CL	GH	IA	FO	CL
15 month	1/5	0/5	4/5	1/5	0/1	0/1	1/1	0/1	0/2	0/2	1/2	1/2	1/3	0/3	2/3	1/3
20 month	3/7	0/7	4/7 ^a	3/7	3/5	1/5	4/5	0/5	2/3	0/3	3/3	0/3	1/3	0/3	3/3 ^a	0/3
25 month	2/5	2/5	1/5	2/5 ^b	0/4	3/4 ^a	1/4	0/4	1/1	0/1	1/1	0/1	0/4	3/4	1/4	0/4
Total:	6/17	2/17	9/17	6/17	3/10	4/10	6/10	0/10	3/6	0/6	5/6	1/6	2/10	3/10	6/10	1/10

GH = Granulosa cell hyperplasia IA = Inactive ovary FO = Predominantly follicles
 CL = Predominantly corpus luteum

^aOne follicular cyst

^bOne corpus luteum cyst

Figures (shown below):

(2A-D) Hematoxylin and eosin (H&E) staining of the epithelial cells lining the vagina (X100).

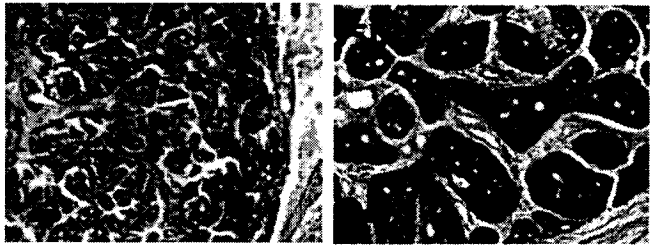
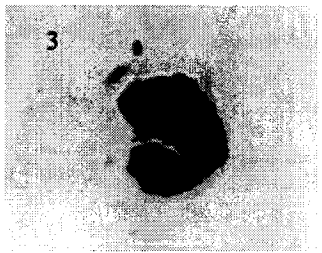
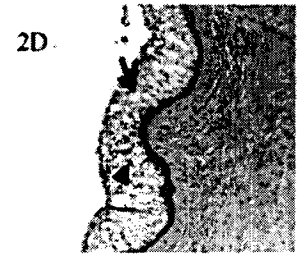
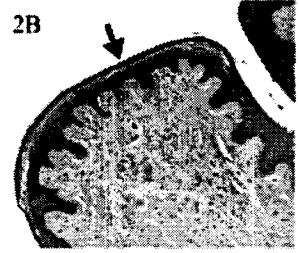
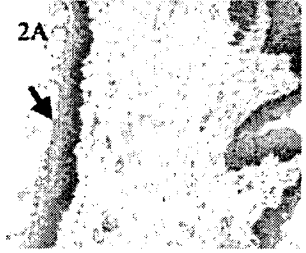
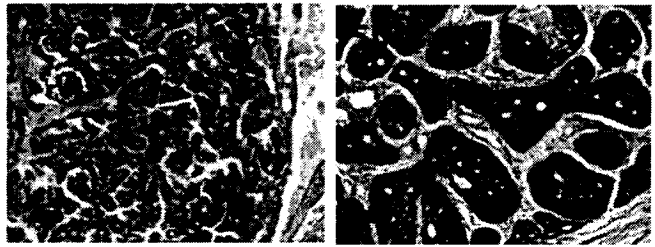
(2A) Proestrus: notice the early formation of the keratinized squamous cells (arrow). **(2B)** Estrus: characterized by the presence of keratinized squamous cells (arrow). **(2C)** Metestrus: notice the presence of stratified squamous cells with no keratinization, and the presence of mild neutrophils. **(2D)** Diestrus: characterized by the presence of many neutrophils (arrowheads) and foamy epithelial cells (arrow).

(3): Endometrial-type cells seen in the vaginal smears from Tamoxifen-treated individuals, characterized by a ball-like structure, high nuclear Cytoplasmic ratio, and hyperchromatic nuclei (X400).

(4): Ovary. Notice the mild hyperplastic granulosa cell layer (arrow) (X50).

(5A): Solid and cribriform pattern of mammary carcinoma is a pattern found more commonly later in life following irradiation during Tamoxifen chemoprevention.

(5B): Papillary carcinoma, the most common histological pattern seen in early carcinomas that develop following irradiation.



COLLABORATIONS:

The Radiation Effects Team currently collaborates with more than nine investigators on research activities or proposals to five agencies: R. Maurer (JHU Applied Physics Laboratory), V. Redeka (BNL), F. Cucinotta (Johnson Space Center), V. Pisacane (U.S. Naval Academy), A. Rozenfeld (CSIRO/JSC), J. Shapiro (Uniformed Services University of the Health Sciences), M. Fenech (CSIRO), Eleanor Blakely (UC LBNL), Gerda Horneck [DLR (Germany/NASA)].

GRANTS AND PROPOSALS					
	TITLE	PRINCIPAL INVESTIGATOR	AGENCY	DATE SUBMITTED	OUTCOME
	Radiation and Weightlessness Effects on Bone Marrow Cells	Jay R. Shapiro, Ph.D.	USUHS of DoD	1/31/02	Reviewed. Scored below funding cut-off
	Automated Non-invasive DNA Damage Measurements in Astronauts	Michael Fenech, Ph.D. and John F. Dicello, Ph.D.	NASA	2/02	Reviewed by astronauts' physician. Undergoing further refinement.
	Imaging & Localizing Device Using the Barkhausen Effect	John F. Dicello, Ph.D.	NIH	4/1/02	Award granted 9/25/2003
	The Risk of Cancer in a Rat Model: Genetic, Cytogenetic, and Abscopal Factors at Low Doses	John F. Dicello, Ph.D.	DOE Office of Biological and Environmental Research (OBER) and NASA	4/16/02	Reviewed. Scored below funding cut-off.
	Radiation-induced Breast Cancer: Detection of Benign and Malignant Lesions in Rat Model	Jerry Williams, Ph.D.	DoD	5/16/02	Rejected
	MIDiN (Microdosimetry investigation) or Microdosimetry of the Earth's Radiation Environment	Vincent L. Pisacane, Ph.D. - USNA	U.S. Naval Academy	5/17/02	Awaiting comment.
	Nonhazardous Early Detection and In Vivo Functional Imaging of Benign and Malignant Tumors	John C. Murphy, Ph.D.	DoD (BCRP)	6/11/02	Rejected
	Advanced Network Infrastructure Technology in Health and Disaster Management	John F. Dicello, Ph.D.	National Library of Medicine	6/14/02	Submitted BAFO – not funded
	Microdosimeter Engineering Model for Spacecraft Experiment	Vincent L. Pisacane, Ph.D. – USNA	NASA Johnson Space Center	7/24/02	Funded

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ORAL PRESENTATIONS

Presentation by John F. Dicello. is based upon material presented in the article, "The Risk of Mammary Carcinogenesis for Sprague-Dawley Rats Irradiated with Low Doses of Energetic Gamma Rays, Protons, and Iron Ions," Dicello J.F., Cucinotta F.A., Gridley D., Mann J.F., Moyers M.F., Novak G.R., Piantadosi S., Ricart-Arbona R., Simonson D.M., Strandberg J.D., Vazquez M.E., Williams J.R., Zhang Y., Zhou H., and Huso D.L., submitted to *Radiation Research* and currently under revision. Bioastronautics Investigator's Workshop, Galveston. (Jan 14, 2003)

Particle radiation - induced genetic damage in transgenic mice, presented by Polly Chang on work of Chang P., Bakke J., Lin S., and Merchant M., Bioastronautics Investigator's Workshop, Galveston. (Jan 12 - 15, 2003)

Imaging & Localizing Device Using the Barkhausen Effect-poster presentation by John F. Dicello, based on research of Dicello, J.F., Von Gutfeld R.J., Ziegler J.F., Ziegler M., and McAllister S., Biomedical Imaging Research Opportunities Workshop in Bethesda, Maryland, (January 31 - February 1, 2003)

Comparison of Preliminary Results for the Risk of Mammary Carcinogenesis in the Sprague-Dawley Rat with Previous Experimental Studies. Presentation of John F. Dicello based upon work of Dicello, J.F., Cucinotta, F.A., Gridley, D., Mann, J., Moyers, M. F., Novak, G. R., Piantadosi, S., Ricart-Arbona, R., Simonson, D. M., Strandberg, J.D., Vazquez, M., Williams, J.R., Zhang, Y., Zhou, H., and Huso, D. 14th Annual Space Radiation Health Investigators' Workshop, League City, Texas, (April 28-30, 2003)

Factors Observed to Modify Mammary Carcinogenesis from Low Doses of Low and High LET Radiations in the Sprague-Dawley Rat. Presentation of John F. Dicello based upon work of Dicello, J.F., Mann, J., Karim, B. O., Christian, A., Eyabi, P. O.¹, Khan K. A., Landolfi, A., Lomabard, P., McAlonis, M., Novak, G. R., Qui, W., Ricart-Arbona, R., Simonson, D. M., and Huso, D. 12th International Congress of Radiation Research, Brisbane. (August 19, 2003)

Microdosimetric consequences of recent in-vivo results for carcinogenesis. John F. Dicello. Keynote Speaker, 2nd International Workshop on "Micro-Mini Dosimetry and its Applications" at World Congress on Medical Physics and Biomedical Engineering. Sydney. (August 26, 2003)



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I. ABSTRACT

The Smart Medical Systems Team (SMS Team) is one of four new teams of the NSBRI, and is now far enough along in its research to begin making significant advances in research and measurable progress toward countermeasure development. At the present time, there are nine projects headed by eight principal investigators. Five projects (led by Drs. Crum, Davies, Klemmpner, Soller and Sutton) address research and development of novel biometric sensors that are lightweight, portable, low power, non-invasive and unobtrusive. These projects have applications for physiological and medical monitoring of astronauts, as well as for the assessment of countermeasures (CMs) that potentially diminish the deleterious effects of long duration space travel. One project (led by Dr. Putcha) develops a novel pharmacological drug delivery system for near term countermeasure administration, while another project (led by Dr. Crum) develops a revolutionary new form of non-invasive surgery. A NASA echocardiographic resource project (led by Dr. Thomas) is supported by the SMS Team, and is jointly supervised with the Cardiovascular Alterations Team. Three projects (led by Drs. Klemmpner, Sutton and Thomas) develop "smart" algorithms for minimal user evaluation and interpretation of real time physiological and medical data. A project added only recently, 5/03, seeks to train astronauts in the techniques of minimally invasive diagnosis and therapy and already has a flight qualified experiment. All of the projects fit within the strategic plan of the SMS Team for NSBRI CM development (sections IIIC and IIID).

Research on the SMS Team aligns itself most closely, albeit not exclusively, with the clinical capabilities category of the Critical Path Roadmap (CPR; <http://criticalpath.jsc.nasa.gov>). Seven of the projects (headed by Drs. Crum, Dulchavsky, Klemmpner, Soller, Sutton and Thomas (x2)) relate to Trauma and Acute Medical Problems (risk #43), which is only one of four Type I, or highest level, risk factors for long duration space missions. In the development of CMs to diminish risk #43, and other significant biomedical risks, the SMS Team has laid out a strategic plan for the development of advanced, integrated and autonomous systems for astronaut health assessment, maintenance and medical care. This plan has initiated, and continues to foster, collaboration with NASA flight surgeons and other medical operations personnel and biomedical researchers affiliated with the NSBRI, Johnson Space Center (JSC), Ames Research Center (ARC), the Jet Propulsion Laboratory (JPL) and NASA Headquarters. The focus of the plan has been at intermediate Countermeasure Readiness Levels (CRLs = 2 to 7), and linkages to NASA programs in medical systems at lower and higher CRLs. One project, headed by Dr. Dulchavsky is already flight qualified and data are presently being collected.

Within the first two years of the SMS Team, significant advances have been made to coordinate research projects and efforts to provide added value. New intra-team (e.g., Drs. Davies and Thomas) and inter-team (e.g., Drs. Soller and Cabrera [Integrated Human Function Core]) NSBRI collaborations have formed. New collaborations between the SMS Team and JSC flight surgeons (e.g., Drs. Strangman, Sutton and Marshburn (JSC)) and researchers (e.g., Drs. Klemmpner and Pierson (JSC)) have developed. Furthermore, a new start-up company has been formed in Seattle, has licensed IP from the University of Washington, and plans to implement technology developed by Dr. Crum's project. Dr. Dulchavsky, recently added to the SMS Team, has stimulated interest from Drs. Crum, Thomas and Putcha as potential collaborators. Regular Team telecons have taken place and specific discussions of intra-team integration have occurred, with several joint efforts contemplated, as well as collaborative interactions with JSC. A SMS Team Retreat was held one day in advance of the Bioastronautics Investigators' Workshop on January 12, 2003. A second retreat is planned, after a determination of which projects will be renewed, sometime in the Spring of 2004.

II. INTRODUCTION

A. Team Objectives

The SMS Team aims to take a leadership role in the research and development of advanced, integrated, and autonomous systems for astronaut health assessment, maintenance and medical care. This includes the delivery and evaluation of medical interventions and other CMs that reduce the deleterious effects of space travel and enhance the overall well being of astronauts. In achieving this goal, it is anticipated that there will be significant impact and applications for earth-based health and medical care. Early progress of team members also show a significant potential for technology transfer to the commercial sector, with two companies recently formed, and negotiations underway to start a third. A major sub-objective of this Team is to develop Commercial Off-the-Shelf (COTS) technology that would could be utilized by NASA to treat problems in space medicine.

B. Health Concerns and Hazards

Health problems associated with space travel may be related to the effects of microgravity, radiation and other risks to the body that are particular to space flight, but they may also be independent of these effects. Medical problems may arise in association with a given demographic population or as a result of a toxic environmental exposure. Moreover, complex interactions may result in alterations and disorders presenting and/or responding differently in a microgravity environment relative to earth. The unique medical circumstances, requirements and limited health care resources in space pose challenges and opportunities for new strategies of physiological monitoring, medical diagnosis and treatment.

In-flight medical events are not uncommon. On STS-1 through STS-89, 98% of crew members reported medical events, excluding space motion sickness (R. Williams, NASA HQ, personal communication). In total, 1867 separate events were logged (1613 men, 254 women), with 141 (7.6%) being due to injury. It is estimated that the risk on the ISS of a significant event, equivalent to one requiring an emergency room visit or hospitalization, is between 1-3 events per annum. The risks increase for long duration space flight and for older crew members. In the Russian space program, two evacuations have been precipitated by medical conditions; in both cases, the entire crews returned.

Given the importance of maintaining crew health, and since medical events can seriously impact astronauts and missions, the CPR ranks Trauma and Acute Medical Problems (risk #43) as one of the four Type I (most severe) risks. Toxic Exposure (risk #44), and Altered Pharmacodynamics and Adverse Drug Reactions (risk #45), are Type II risks. Illness and Ambulatory Health Problems (risk #46), Decompression Sickness Complicated by Microgravity (risk #47), and Post-landing Rehabilitation (risk #48) are Type III risks.

C. Topics to Address

The SMS Team recognizes that to achieve its objectives (section IIA), it must (a) utilize a team approach within the context of the NSBRI CM driven mission, (b) coordinate and collaborate with other NASA efforts in space and critical care medicine, (c) emphasize research that leads to testing and monitoring of physiological functions and CM effectiveness in healthy astronauts (i.e., link the SMS Team to other NSBRI teams and promote CM research with broad

utilization), as opposed to emphasizing trauma and acute problems only, and (d) develop alternative approaches to medical care, given that resources are limited, there may be no M.D. in flight and communications to earth are limited and delayed.

The NSBRI assigned the SMS Team a mandate to develop innovative, possibly revolutionary, techniques for medical monitoring, diagnosis and treatment. To achieve these goals, several infrastructural needs have been identified. These include:

- New types of biometric sensors
- Novel medical and surgical techniques
- Robotic medical assistance systems
- Advanced drug synthesis and delivery systems
- Smart algorithms for medical data systems
- Automated decision support for training and care
- Systems engineered platforms for sensor, algorithm and effector integration

Additionally, it has been necessary to develop a strategic plan to link projects together, and this is described section IIIC.

III. RESEARCH PROGRAM STRUCTURE & DESIGN

A. Program Structure and Interactions

As stated in section IIA, the SMS Team objectives are broad and ambitious. However, the research program needs to be focused in order to ensure clear scientific progress towards CM development. To this end, specific synergistic relationships either exist or are being developed among team projects, as well as between SMS Team projects and other NSBRI and NASA projects. These relationships are summarized in Fig. 1.

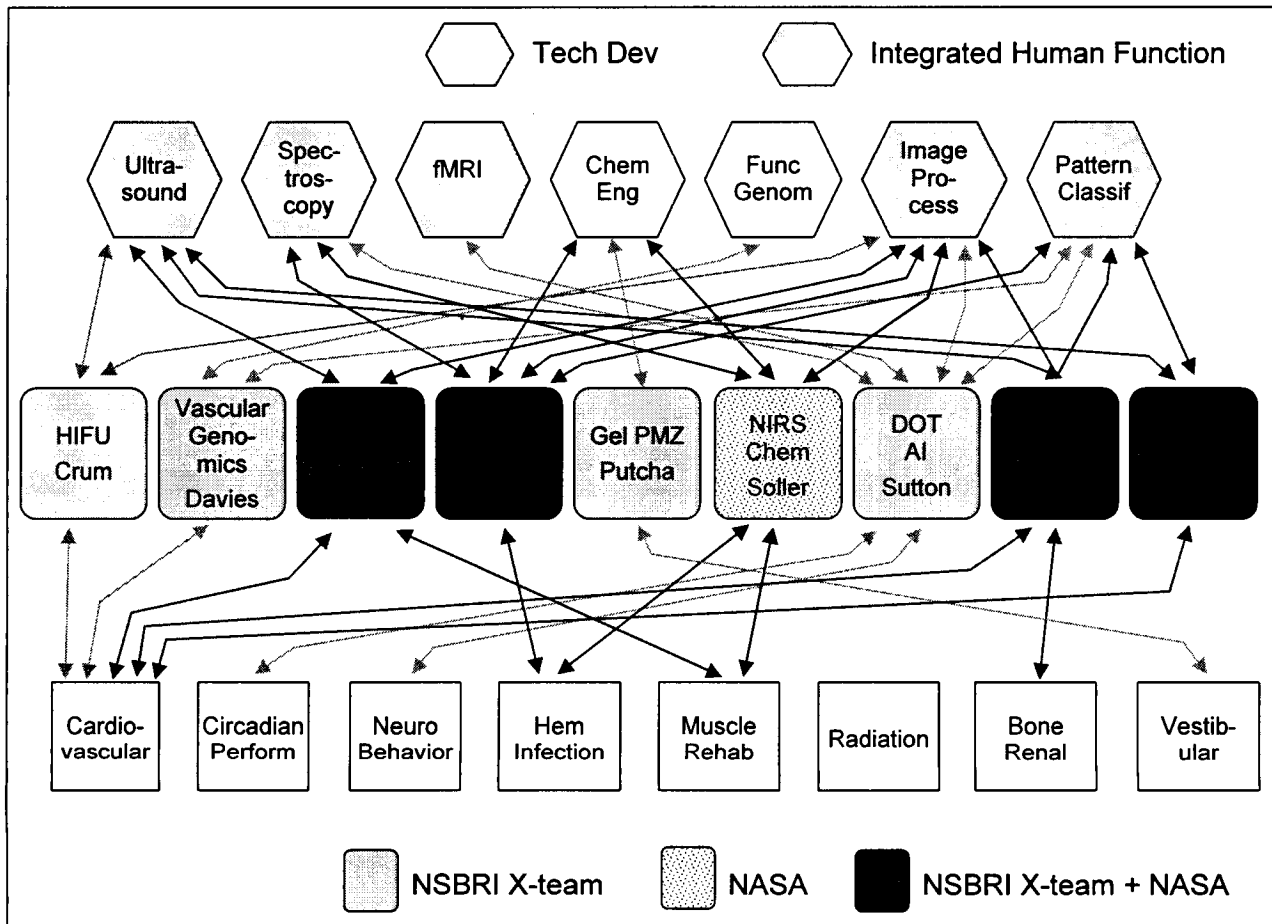


Figure 1. SMS Team project interactions showing relationships within and between NSBRI teams

In Fig. 1, each of the nine SMS Team projects is represented along the middle row. The projects are coded to depict (a) NSBRI cross-team interactions (Soller with Cabrera (Integrated Human Function Core)), (b) NASA interactions (Davies with Luzod (ARC); Putcha (JSC); Sutton/Strangman with Marshburn (JSC); Crum with Hines at Ames), (c) NSBRI cross-team and NASA interactions (Klempner with Fox (Immunology, Infection and Hematology Team) and Pierson (JSC); Thomas with Cohen (Cardiovascular Alterations Team) and JSC); and Dulchavsky with JSC.

The bidirectional arrows in Fig. 1 represent relationships (a) among projects within the SMS Team and (b) between SMS Team projects and the other NSBRI teams. These relationships are broken down into two main categories: Technology; and Physiological Systems and Effects. Across the top row, interactions between SMS Team projects and the Technology Development and Integrated Human Function Core (this unit is no longer a formal Team; rather they have been integrated into the NSBRI Core, but the PIs from this effort are still active) are shown. These interactions correspond roughly to experimentation (Technology Development Team) and theoretical or modeling (Integrated Human Function Core) interactions. Arrows pointing to particular boxes *from* SMS Team projects *to* boxes in the upper row show how SMS Team projects contribute to NSBRI developments in specific domains on *other* teams. For example, the Klemptner, Soller and Sutton projects all develop novel spectrographic devices that complement one or more projects being developed in the Technology Development Team (specifically, projects headed by Potember and by Maurer).

Arrows that originate *from* boxes in the upper row of fig. 1 and project *to* SMS Team projects represent links among projects *within* the SMS Team. The relationships are incomplete and are evolving, sometimes with added benefit to the overall NSBRI scientific program. For example, ultrasound technologies link projects by Crum and Thomas, although Thomas' projects do not develop hardware; similarly, the recent addition of Dulchavsky to the Team introduces a large number of additional intra-Team interactions, e.g., Crum and Dulchavsky are both interested in ultrasound guided therapy; Thomas and Dulchavsky are both interested in ultrasound-based diagnosis. The functional magnetic resonance imaging (fMRI) aspects of Sutton's project adds to the (non-functional) MRI developments in the Technology Development Team; hence the half shaded box in Fig. 1. The chemical engineering and functional genomic and proteomic approaches on the SMS Team complement other core technology developments within the NSBRI program.

Bidirectional arrows between the boxes representing the SMS Team projects and the system teams along the bottom row of Fig. 1 work similarly to those just described. There is synergy with every system team, especially the Cardiovascular Alterations Team. There is also an emerging emphasis on brain and neurobehavioral alterations within the SMS Team (Sutton/Strangman project). At present, there is no synergy with the Radiation Effects Team, although there is scientific overlap with that team.

B. Program Strategy

While the previous section outlines the relationships among projects, it does not describe the design of the SMS Team to address research problems and develop countermeasures. To understand how team R&D might lead to deliverables for eventual implementation for flight, a strategic plan is required. To achieve this goal, a high level description of the system for health and medical monitoring, as well as interventions, that are currently in place was constructed. In this system, the astronaut and environment are handled in similar ways, since space medicine is effectively a branch of aerospace or environmental medicine. Sensors monitor the environment and astronaut, and after calibration, signal conditioning and processing, data are either stored and/or relayed to earth. Ground based personnel oversee, in coordination with the astronaut, flight surgeon and possibly the PI if appropriate, any treatment or countermeasure that is administered. There is limited autonomy and ability to assess deconditioning effects in space. Countermeasure modification and medical care delivery is severely limited.

In collaboration with NSBRI, NASA JSC, ARC, JPL and other personnel, a strategic plan for the SMS Team was developed during the winter and spring months of 2001. The objectives were to develop a schematic that (a) characterized a “smart medical system”, (b) linked NSBRI SMS Team research and countermeasure development to basic research, industry, space hardware and medical operations, (c) provided a format to map current projects within the SMS Team onto a system prototype, both at the component level and at the level of the system itself, and (d) allowed for the identification of gaps in the SMS Team program.

In this plan, information from the environment and astronauts is sensed by a suite of small, lightweight, low power, portable, non-invasive, unobtrusive, intelligent sensors with pattern recognition capabilities. These sensors feed automatically analyzed, rather than raw, data into decision making algorithms, that also have cognitive input from the astronauts themselves. There is a model of the system, which is where the Integrated Human Function Core collaborations fit in the scheme. The model not only (a) assesses input from multiple sources, but it (b) pre-plans notification for onboard alarms and information transfer to the ground, (c) looks at contingencies and outcomes for effectors and treatments prior to the administration of CMs, (d) assesses the effectiveness of treatments and CMs, (e) monitors consequences of actions and CMs, and (f) interfaces with models for pattern recognition and analog hardware learning. Since feedback loops exist which are independent of external and M.D. control, the system is, in principle, autonomous. Moreover, the design is achievable, to varying degrees, and proposes a revolutionary new health care system for space, which is central to the initial research charge assigned to the SMS Team. Of course, assembling such a complex system requires first the development of system components that permit later system integrations to occur. Much of the present work involves component development, but as these components mature and function robustly, the integration the various components into a fully functional integrated system can occur. In summary, the main points are as follows:

- Enhanced small sensor platforms with pattern recognition and wireless capabilities
- Adaptable system of systems for sensor integration
- Algorithms and models for human assisted monitoring, CM assessment and decision making
- Common platforms for sensing and CM delivery

C. Countermeasure Development

To see that the program strategy of the SMS Team is aligned with the mission of the NSBRI, each project in section IIIA, along with its relationships to other projects (section IIIB), was mapped onto the strategic plan set forth in section IIIC. The CM development plan for the SMS Team was then identified. It includes several types of measures, as outlined below.

Specific countermeasure developments include:

1. Exercise

Soller: non-invasive tissue and blood chemistry measures for physiological monitoring and assessment of exercise effectiveness applicable across ethnic races

2. Pharmacology

Putch: novel drug delivery system, with first application to intranasal promethazine HCl to reduce space motion sickness

3. Training

Thomas: ultrasound resource to train naïve users in medical image acquisition, with multi-systems applications (e.g., cardiovascular, bone, renal)

Dulchavsky: training of astronauts to use minimally invasive sensors and therapy devices both to detect and to treat conditions that are difficult to treat in microgravity

4. Performance Adjustments

Sutton/Strangman: non-invasive assessment of brain function under cognitive load and sleep disturbance to adjust performance expectations

5. Environmental Manipulation

Klempner: real-time assessment of distributed microbial environment for early detection and manipulation of significant alterations; sensors applicable for a broad range of environmental monitoring and manipulation

6. Surgery

Crum: non-invasive use of ultrasound for diagnosis and treatment of injury

Dulchavsky: use of minimally invasive systems for diagnosis and therapy

7. Functional Genomics

Davies: functional genomic approaches to address vascular changes in microgravity

8. Adjunctive Developments to Other CMs

Klempner, Soller, Sutton, Thomas:
suite of passive continuous physiological monitors and algorithms to identify the need for, and efficacy of, specific CMs, including those related to medical care

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

A. Accomplishments

Although SMS Team is still a relatively new start, considerable progress has been made. From a general perspective, the SMS Team has developed a strategic plan and coordinated research team efforts that (a) successfully interfaces with other programs within the NSBRI and NASA medical operations, and (b) has high potential impact for NASA through CM development to reduce the risks associated with long duration space travel. While funding interruptions have delayed and disrupted steady progress, effective synergies have been formed and progress has been made on each of the projects. There was a PI retreat in April 2001, and a second one in January 2003; after renewal evaluations are made, the new Team members and leaders should probably have a retreat in the late spring or early summer; regular telecons, multiple site visits among investigators, and education and evaluation of previous and current medical CMs have occurred. The team strategy was expanded from its initial focus on medical care to research on physiological monitoring, algorithms and CM effectors and assessment. More specifically, the main findings and accomplishments during the past year for each project are summarized below.

PRINCIPAL INVESTIGATOR:	Lawrence Crum, Ph.D.
PROJECT TITLE:	Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care

PURPOSE

The principal objective of this project is to develop an image-guided ultrasound therapy system for mission critical care. -- In long-duration space flight, a number of medical situations could develop that if not adequately addressed would result in mission failure. For example, although gravity is significantly reduced in space, inertia is not, and the collision of an astronaut with a heavy object could result in blunt internal trauma and its often associated internal bleeding. In addition, as recent experiences in Antarctica demonstrate, medical conditions that require some form of surgery may well appear without warning, even when extensive pre-screening is undertaken. This proposal addresses three specific risks as described in the CPR as well as in the most recent NSBRI Strategic Plan (draft of 5/5/03); viz., Risk No. 43--Trauma and Acute Medical Problems; Risk No. 23--Carcinogenesis Caused by Immune System Changes; Risk No. 38--Carcinogenesis Caused by Radiation

BACKGROUND

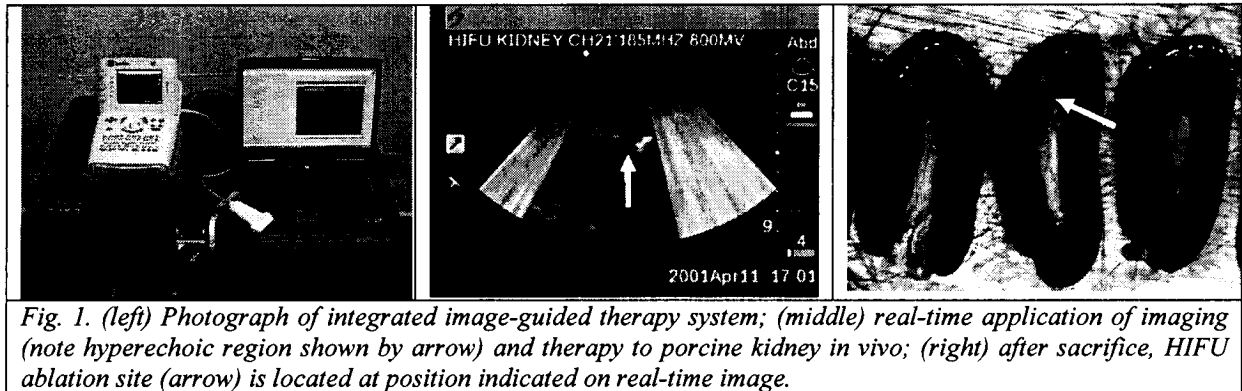
Currently, medical devices are being developed that utilize high-intensity focused ultrasound as a noninvasive method to treat tumors and to stop bleeding (hemostasis). The primary advantage of ultrasound is that it delivers intense heating to a millimeter to centimeter size region deep in the body without damaging intervening tissue such as the skin. The second advantage is that ultrasound can simultaneously be used to monitor the treatment.

RESULTS

Four key development have been accomplished that are on our critical path: (1) A small portable image-guided HIFU system has been constructed and tested (See Fig. 1, laptop is optional); (2) A new power supply was developed and tested that reduced the system weight by over 50%. (Through a related NIH SBIR grant, a commercial partner is now marketing a version of this compact power supplies for HIFU systems.) (3) Significant strides were made toward optimizing performance. Most importantly, we demonstrated that doubling the acoustic amplitude of the source cuts the treatment time by more than 1/2. In fact, in some experiments, the energy required to denature proteins was reduced by a factor of three when the pressure was increased by the 1.4 ($\sqrt{2}$); and (4) A provisional patent application was submitted for a circuit to permit real-time synchronization of the HIFU therapy with any ultrasound imaging device.

Four other key findings are also important to our SMS project: (1) Our group discovered that HIFU produced both hemostasis and pneumostasis in the punctured lung. Although we had shown we could stop bleeding in the liver, spleen, and peripheral vessels, air in the lungs which reflects ultrasound made trauma treatment in the lung an unknown. We were delighted to discover that the lung is sealed more quickly than liver or spleen; (2) Our group developed a new algorithm to monitor HIFU therapy using RF data acquired from an ultrasound imager; (3) In collaboration with Prof. Y. Matsumoto (University of Tokyo), we discovered that we could use HIFU to comminute kidney stones; and (4) we established a new and innovative course with

Prof. L. St. Pierre of the Industrial Design department at UW (UWID). ID students learned about HIFU technology, researched areas of application, and refined and developed design proposals that would suit the technology to the application. The project has also drawn the attention of the Dean of the College of Arts and Sciences, and members of the professional design community.



IMPACT AND IMPLICATIONS

- The components in Fig. 1 now weigh less than 10 kg, down from more than 30 kg a year ago and 40 kg at the start of the project. They can be packaged in a single metal chassis (the laptop computer is no longer required) and operate with the Philips HDI-5000 ultrasound imager on the ISS or whatever imager is chosen for long duration missions (i.e., a Sonosite imager such as used in the SMS project led by Dr. J. Thomas). The new circuit makes it possible to provide therapy while imaging in Pulsed Wave Doppler mode.

- We have established a protocol that would provide HIFU in a continuous, spatially stream (by moving the transducer) of short-duration, high-amplitude pulses, a procedure that permits us to treat large areas rapidly without complications from cavitation. This approach includes the induction of both hemostasis and pneumostasis to a damaged lung. These findings expand our trauma care capabilities, improve therapy efficiency, and minimize the power required by the device.

- We have also developed a fully integrated laboratory system to capture RF data and a new algorithm to monitor lesion formation by change in attenuation of ultrasound in tissue. The system is more sensitive than standard B-mode imaging and can be used to assess the completeness of a volume treatment after cavitation bubbles have been reabsorbed by the body.

- Lastly, we are expanding the ability of our existing Image-Guided-Therapy device to image and comminute renal calculi that might form in an astronaut as a result of mineral depletion [Renal Stones Formation, Risk No. 12 on the Bioastronautics Critical Path Roadmap].

RESEARCH PLAN FOR UPCOMING YEAR

The system in Fig. 1 is to be packaged in one chassis and tested in laboratory animal studies for the induction of hemostasis and tumor ablation. The circuit for synchronization will be converted from breadboard to a printed circuit board. Using the image-synchronization technology that we have developed, we plan to integrate a Pulsed wave Doppler ultrasound imaging capability with the therapy transducer into a single unit. Therefore, only one transducer needs to be used to provide both imaging and therapy. In a continuing effort to optimize our therapy protocol, we have developed a dual-frequency transducer that can produce both low and high frequency outputs. Preliminary evidence shows that the low frequency transducer increases cavitation and

thus expands the induced HIFU lesion size. We plan a careful study to determine if mixing two frequencies can accelerate hemostasis on large bleeds by expanding the focal region. Supplemental grant applications have been submitted for a study of kidney stone comminution with ultrasound, as well as an SBIR proposal to work with an industrial partner to investigate what aspects of our HIFU technology can be commercialized.

CONCLUSIONS

We have demonstrated that a device that produces High Intensity Focused Ultrasound (HIFU) can be combined with a device that provides ultrasound imaging to produce a duplex system that can both image a particular condition of interest and provide therapy to that region. "Image-Guided Therapy" provides enormous potential for the treatment of a variety of medical conditions, particularly those associated with blunt trauma and internal bleeding. In addition, we have demonstrated significant progress toward making the system both lightweight and portable.

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PRINCIPAL INVESTIGATOR: Peter Davies, Ph.D.
PROJECT TITLE: Vascular Genomics in Gravitational Transitions

AIMS

When changes in the biomechanical environment of the circulation occur, blood vessels undergo well-orchestrated structural and metabolic remodeling to restore optimal function. We propose that this remarkable adaptive ability lies at the center of orthostatic intolerance exhibited by most astronauts on return to earth's gravitational field after modest-to-long periods in microgravity. We are therefore mapping gene expression (transcription profiling) of the different vascular steady states exhibited *in vivo (mouse)* in simulated hypergravity and microgravity, and the transitions between them, in order to design better countermeasures for undesired vascular consequences in long-term space flight. In particular, the transition to hypergravity will simulate the effects experienced by astronauts upon return to a significant gravitational field (Earth, Mars) following adaptation to long periods of microgravity. The studies will generate a reference genomics database identifying gene expression changes in the arteries, heart and lungs induced by gravitational shifts and mining of such databases will provide a guide to potential countermeasures to offset deleterious effects.

KEY FINDINGS

(i) During the first year we refined the antisense RNA techniques necessary to amplify RNA from small numbers of cells with high fidelity. This became necessary when it was apparent that no literature existed for a rigorous test of the protocols required in the mouse experiments. In a model experiment, vascular cells were stimulated with the cytokine TNF for which a small number of genes are known (through conventional Northern analyses) to change. RNA from the same pool was analyzed by microarray with and without amplification. Sophisticated bioinformatics analysis of 13,800 genes was performed. The data from unamplified and amplified RNA were analyzed for fidelity, sensitivity and utility. The expected prominent changes in known genes were detected in both groups with high retention of accuracy, an essential requirement for the proposed *in vivo* gravity experiments. An interesting additional and unexpected finding is that RNA amplification increased the detection rate of genes whose differential expression was just below a significance threshold in the unamplified assay i.e. greater sensitivity of detection of differential gene expression conferred by the linear amplification techniques employed. Most important, these differences were confirmed by real-time quantitative PCR of unamplified RNA. This work was published in the journal *Physiological Genomics* in April 2003 (reference 1). The studies were a prerequisite for the gravitational experiments because no such analysis existed that rigorously evaluated the accuracy of the transcription profiles arising from amplification of small amounts of blood vessel.

(ii) In extending the RNA amplification techniques we next addressed differential vascular cell gene expression in two sites in the aorta of the normal adult pig. Endothelial cell mRNA was isolated from two regions of the aortic arch characteristic of disturbed flow (pro-atherogenic) and undisturbed flow respectively. RNA from paired sites in individual aortas (n=8) was isolated, linearly amplified, reverse transcribed, and cDNA was hybridized to microarrays custom-prepared from the University of Toronto human cDNA cardiovascular database (~8000 genes) plus several thousand proprietary Incyte clones. Bioinformatics analyses identified expression patterns in the disturbed flow region indicative of an antioxidant endothelial profile that may be protective of a pro-inflammatory state. Some genes associated with major mechanisms believed to initiate atherogenesis, eg pro-inflammation, were elevated but the critical adhesion molecules

necessary to initiate inflammation were not differentially expressed in this region, consistent with the absence of any pathology by histological assessment. *This is an intriguing result that demonstrates the power of this approach in identifying the interactions of multiple genes need to be considered in defining atheroprotective or susceptible situations.* As far as we are aware, these are the first high throughput array analyses of arterial endothelial gene expression directly obtained from discrete regions of blood vessel. When compared with several studies that have profiled the effects of different flow treatments on cultured (as opposed to *in vivo*) cells, many differences of gene pathways were noted. This work has been submitted to the journal *Proceedings National Academy of Sciences USA* (reference 2). While these studies were performed with larger blood vessels (porcine) in order to obtain enough lining cells (endothelium), the cell numbers used are comparable to, in fact less than, those we will obtain from whole mouse aorta for the gravity studies. Techniques for the dissection of mouse blood vessels, RNA isolation and amplification has been verified under normal gravitational conditions. These evaluative experiments demonstrate that we can successfully perform the entire sets of protocols from tissue isolation to bioinformatics and gene annotation prior to the gravitational shift experiments at NASA-Ames Research Center.

(iii) At time of writing (early October 2003), the PI is conducting hypergravity and transitional procedures upon 96 mice at NASA Ames using the 24ft centrifuge in close collaboration with Ames staff members Tianna Shaw and Tom Luzod. Arterial, heart, and lung tissues harvested in Oct through November will then be analyzed at Penn. We hope to have preliminary Gravitational Database (GRAV-DB) information (limited to one or two time points only) in time for the NSBRI retreat in January. The molecular biology is demanding and lengthy, and the bioinformatics is complex. We estimate completion of the hypergravity databases by late summer 2004. As in the case of our publications to date, we are taking steps to ensure that the data are openly available to the widest scientific community (see texts of refs 1 and 2 outlining weblinks and online data repositories). This reflects the quality of the Bioinformatics collaboration involved in the project through co-investigator Dr. Chris Stoeckert and Postdoctoral Fellow in the PI lab, Dr. Anthony Passerini.

IMPACT AND IMPLICATIONS

New techniques addressing vascular genomics have been developed, tested, and have withstood critical peer review in leading journals in the field of genomics and biology. We are now implementing them in carefully designed experiments in which gravitational shift is the variable. We expect therefore to be successful in producing a valuable gravitational genomics database by the end of the current funding period (Aug 31 2004).

COMING YEAR PLANS

For studies of hypergravitational changes, the facilities of the Chronic Hypergravity Exposure Centrifuge at NASA Ames are suitable for long-term exposure of mice at 3G to simulate return to earth (or landing on Mars surface) after long term space travel. The current experiments will provide the conditions for database development. Mice will be exposed to micro or hyper gravity for up to 28 days and the effects upon gene expression in the major arterial system, heart and lungs will be measured by the techniques outlined above. Reversal of the adapted condition will also be evaluated on a temporal basis.

PUBLICATIONS

Polacek, D.C., Passerini, A., Shi, C., Francesco, N.M., Manduchi, E., Grant, G., Powell, S.J., Bischof, H., Winkler, H., Stoeckert, C., Davies, P.F. 2003. Fidelity and enhanced sensitivity of

differential transcription profiles following linear amplification of nanogram amounts of endothelial mRNA. *Physiological Genomics* 13:147-156.

Passerini, A., Polacek, D.C., Shi, C., Powell, S.J., Francesco, N.M., Manduchi, E., Grant, G., Chang, G., Pritchard, W.P., Stoeckert, C., Davies, P.F. Differential gene expression reveals coexisting pre-inflammatory and atheroprotective endothelial profiles in a disturbed flow region of the adult porcine aorta. *Proc. Natl. Acad. Sci. USA* Submitted

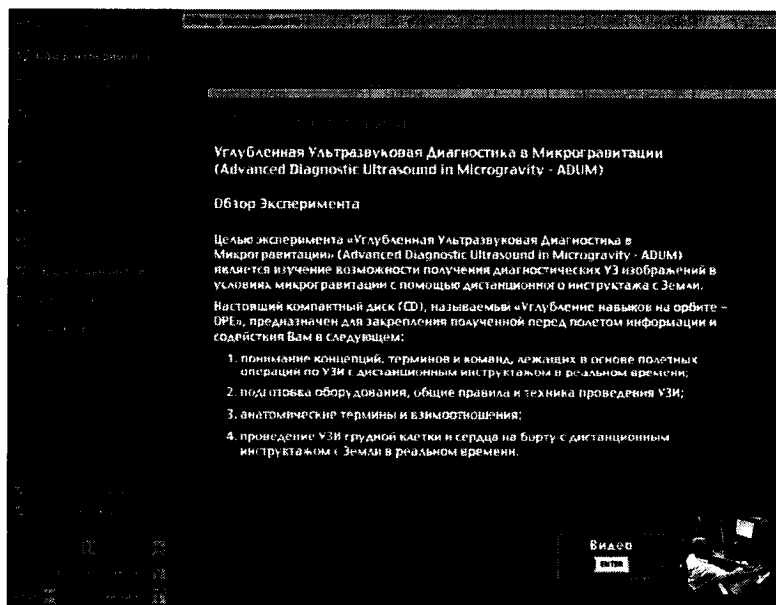
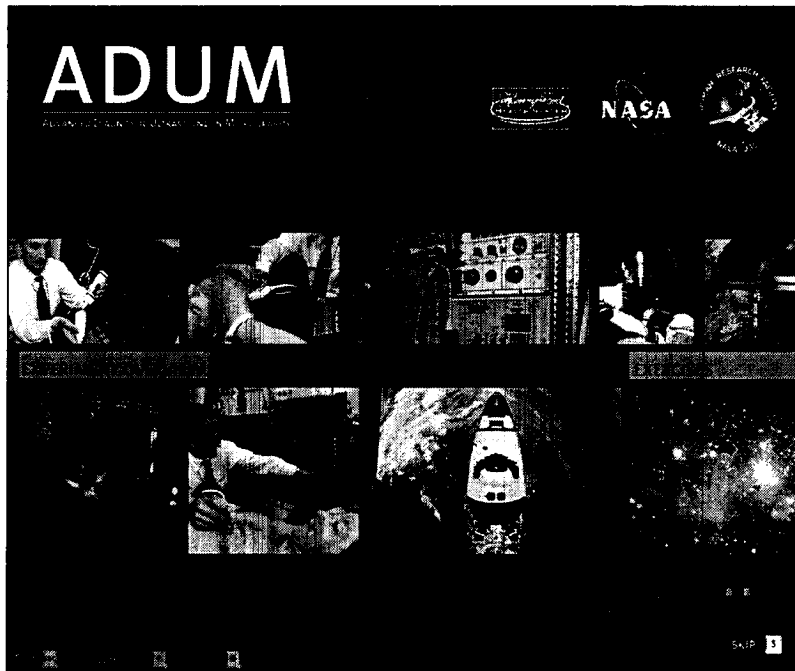
PRINCIPAL INVESTIGATOR: Scott A. Dulchavsky MD, Ph.D. PROJECT TITLE: Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies

Executive Summary: The diagnosis and management of acute health problems in space is problematic due to limited training of the Crew Medical Officer (CMO), human and environmental factors, and a lack of reference of the changes in anatomy, disease presentation, and therapy in micro-gravity. There is no planned radiological capability aboard the ISS further complicating medical diagnosis in space.

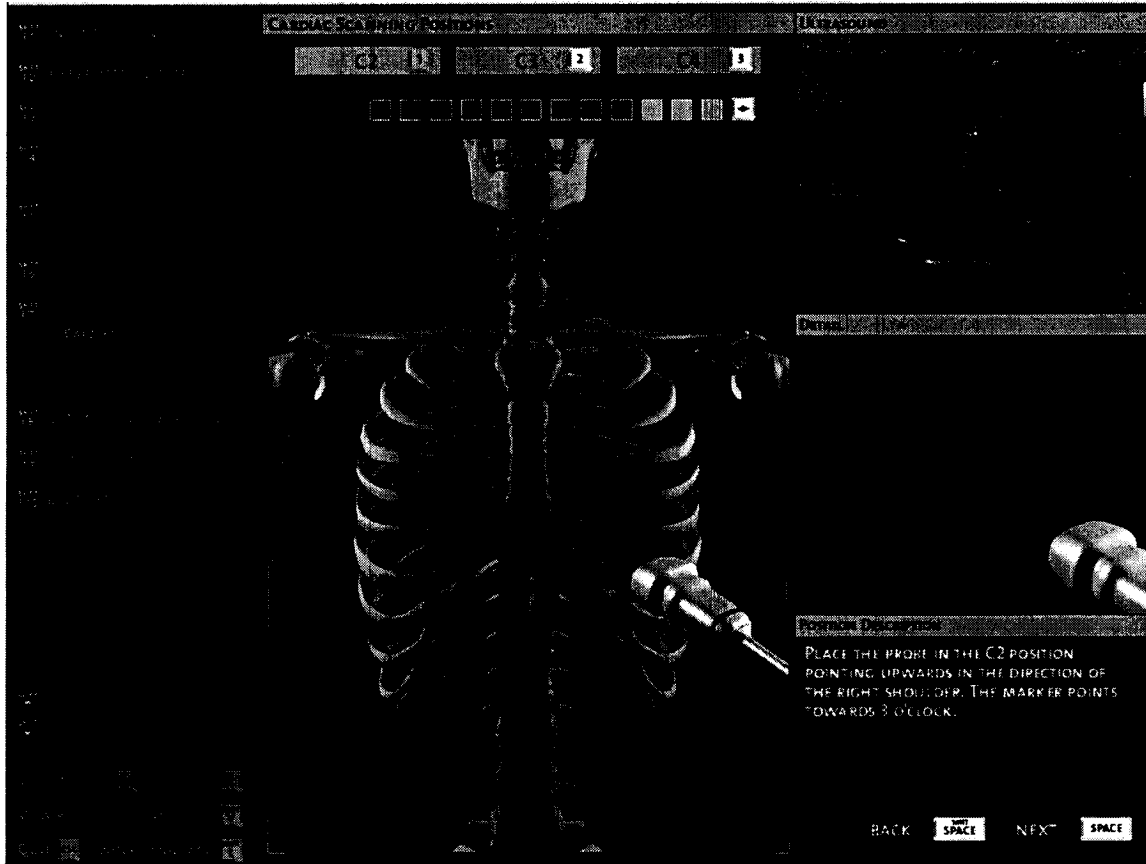
Recent terrestrial investigations suggest expanded clinical applications of ultrasound and laparoscopy with miniature instrumentation which have applicability for aerospace medicine. This proposal will initially determine the diagnostic utility of ultrasound and/or micro-laparoscopy in select health contingencies with high potential mission impact. These diagnostic modalities will then be used to facilitate minimally invasive, definitive surgical therapy of selected contingencies in animal models in ground based and simulated micro-gravity scenarios. Optimal just-in-time training regimens and computer based refresher modules for non-physician CMO's to accomplish these tasks will be developed.

The unique constraints imposed by training and equipment limitations and the space environment require the development of novel diagnostic and therapeutic strategies for crew member health problems including the expansion of ultrasound and mini-laparoscopy. Thoracic ultrasound, initially investigated by NASA as an alternative diagnostic modality for pneumothorax, has proven accuracy in terrestrial and micro-gravity applications and will have wide spread impact in acute care on Earth in the future. Although some of the techniques investigated in this proposal are appropriate only for a micro-gravity environment, the majority of the diagnostic and therapeutic algorithms are readily transferable to terrestrial medicine including rural and military applications. The expanded use of the diagnostic and training modalities described in this proposal, if verified, would provide a significant, clinically relevant advance in space medical capabilities with profound Earth-based ramifications.

Progress: We have developed an interactive, intuitive, multi-lingual CD-ROM based instructional module to facilitate just-in-time training in advanced ultrasound for non-physicians. The program is modular to facilitate rapid changes, uploadable, and functions on any computer platform. Skills enhancement and programmatic negotiation are stored in a searchable executable file to facilitate learning enhancement and future refinements.



The instructional modules utilize state of the art computer rendering to facilitate 3 dimensional anatomic concepts which are crucial to obtaining and interpreting diagnostic quality advanced ultrasound images. Common operator errors and enhancements are included in the tutorials.



We are implementing internet based, remote expert guidance capabilities to assess these training paradigms in minimally trained ultrasound operators. Remote ultrasound experts in Houston will begin investigations using medical students in Detroit as ultrasound trainees in the near future.

PRINCIPAL INVESTIGATOR: Mark S. Klempner, M.D.

PROJECT TITLE: Smart Medical System for Detection of Microorganisms

The project objective is to develop a rapid, non-culture based method to detect, identify, and quantify microorganisms from environmental and clinical samples. The method relies on using highly diverse phage displayed peptide libraries from which phage clones that bind to the surface of various organisms are selected. It is possible to discriminate one organism from another using phage clones expressing different peptides that bind to various organisms with different affinities.

RECENT RESULTS

In this section we will present recent data demonstrating the feasibility of obtaining diverse ligands for surface molecules on spores from *Mir* fungi using phage displayed peptide libraries. Isolates of *Aspergillus niger* and *Penicillium chrysogenum* were provided by Dr. Duane Pierson of the Johnson Space Center. Horizontal culture flasks containing 25ml of Sabouraud Dextrose agar were inoculated with 50 μ l of fungus spores that were stored as a frozen stock in 50% glycerol at -20°C . The flasks were cultured at room temperature for 7-10 days until a lawn of fungus was present. 25ml of PBS pH 7.4 was added and the spores were harvested using a cell scraper. The spore solution was filtered through gauze and suspended in PBS at a concentration of $2.5 \times 10^8/\text{ml}$ and stored at 4°C .

For our initial experiments we used a combinatorial library of random peptide 7-mers fused to the minor coat protein (pIII) of M13 Phage. The displayed heptapeptides are expressed at the N-terminus of pIII. The peptide is followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. The library consists of $\sim 2.8 \times 10^9$ possible 7-residue sequences, amplified once to yield ~ 70 copies of each sequence. For biopanning phage (2×10^{10}) were added to 1ml of spores (2.5×10^7) in PBS, incubated with rotation at room temperature for 60 minutes, microfuged (11,000xg) for 5 minutes and then washed three times with 1ml PBS. The phage were eluted from the spores by the addition of 1ml of 0.2M Glycine-HCl pH 2.2 in 1mg/ml BSA and incubated for 5 minutes, microfuged for 5 minutes and the supernatant was removed and neutralized with 0.15ml 1M Tris-HCl pH 9.1. The eluate containing M13 phage were amplified in *E.coli* ER2738 host strain and the secreted phage were isolated and titered. Biopanning of the amplified phage was repeated twice with spores from the same culture.

The elution from the last "biopan" was titered and individual clones were isolated by excising individual plaques from the agar plate, amplified, isolated and titered. To determine that the individual peptide displaying phage clones (2×10^{10}) bound to spores each was incubated with spores (5×10^7) in 100 μ l of PBS with agitation at room temperature for 30 minutes. Spores were isolated by centrifugation, washed extensively and incubated with a mouse monoclonal antibody directed at pVIII on M13. After washing the spores (with their bound phage and mouse anti-phage antibody) were incubated with anti-mouse antibody conjugated with alkaline phosphatase. After washing and addition of alkaline phosphatase substrate the optical density at 405nm of samples was read in a microplate reader. The specific binding was determined by subtracting the optical density of samples from wells to which phage were added but not spores.

Figure 1 shows representative results from the above procedure when spores from *Aspergillus niger* (left panel in green) or *Penicillium chrysogenum* (right panel in blue) were used. Biopanning with this one 7-mer library we restricted picking individual clones to 200 that demonstrated a spectrum of binding activity for these spores. Of course many more clones with "high", "medium" and "low/no" affinity could be isolated from this library as well as the other libraries that we have used and propose to use in this application.

Phage Clones Binding to Spores

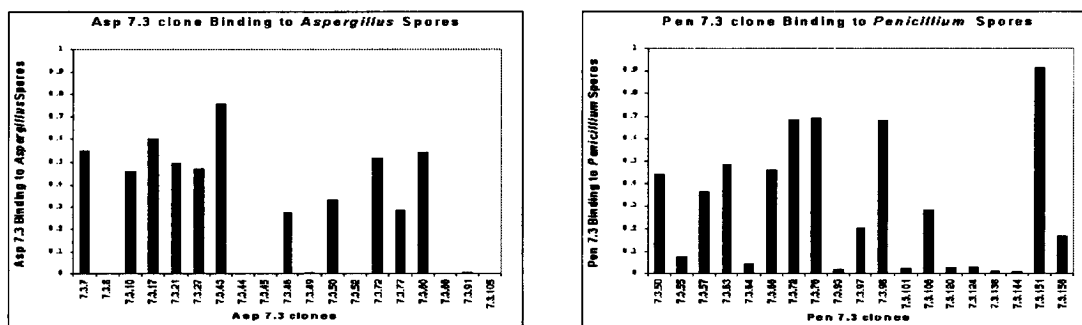


Fig. 1. Representative results of phage binding.

Because there are multiple copies of each phage displayed peptide clone in the incubation mixture it was of interest to determine the redundancy and diversity of the clones that bound to *Aspergillus* and *Penicillium* spores. We selected those clones that bound with highest affinity for sequencing. DNA from individual clones was isolated and sequenced using the -28gIII sequencing primer. The sequence corresponds to the *anticodon* strand of the template. Because TAG stop codons are suppressed by glutamine in ER2738 (*supE*), the strain originally used to produce the library TAG is considered a glutamine codon when translating. In addition to unique phage displayed peptides which bound to *Asp* or *Pen* spores, multiple copies of some phage displayed peptides were isolated (e.g. Asp 7.3.7, 7.3.10., 7.3.17, 7.3.21, 7.3.27) adding confidence to the selectivity of biopanning in this system as it has displayed using cell and protein targets.

To determine if phage displayed peptides with high affinity for *Aspergillus* or *Penicillium* spores could discriminate between and capture them onto a surface, phage from individual clones were bound to flat bottomed 96 well microtiter plates using mouse antibody against M13 p-VIII which was used to first coat the wells. This permitted orientation of the phage allowing peptides displayed on M13 p-III to be accessible. After incubating each well with the different phage clones (2×10^{10}), the wells were washed and mixed populations of *Asp* and *Pen* spores (2×10^6) were added to each well. Spore binding was measured with specific alkaline phosphatase conjugated rabbit polyclonal antibodies that had been extensively absorbed to remove cross reacting antibodies. As shown in Figure 2, phage displayed peptides which had been selected for their high affinity binding to *Asp* or *Pen* selectively bound to and captured the correct spores. A few phage displayed peptides (e.g. Asp 7.3-80 and Pen 7.3-151) bound both *Asp* and *Pen* spores with high affinity.

Phage Capture of *Aspergillus* and *Penicillium* Spores

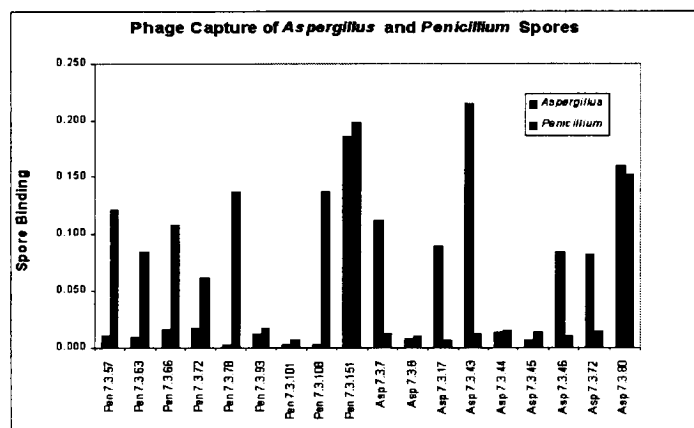


Fig. 2. Illustration of phage capture.

To extend these findings we have also obtained over 200 phage display peptides that differentially bind to Asp or Pen from 2 other libraries (one with 12 linear amino acids (12 mer) and another library with a constrained loop of 7 amino acids flanked by cyteines). We have also begun to express the cognate peptides from the constrained loop peptide library as maltose binding protein (MBP) fusions. We hypothesize that some structured peptides will retain their binding characteristics to the spores and they can be readily attached to amylose coated plates or to a sensor surface using binding via MBP. The system uses the pMAL vectors which are designed so that insertion interrupts a *lacZ α* gene allowing a blue-to-white screen for inserts on X-gal. pMAL-c2 series has an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. pMAL-p2 fusion proteins capable of being exported can be purified from the periplasm. Between the *malE* sequence and the polylinker there is a spacer sequence coding for 10 asparagine residues. This spacer insulates MBP from the protein of interest, increasing the chances that a particular fusion will bind tightly to the sugar residue and leave the expressed peptide oriented and accessible.

In addition to expanding the number of reagent phage clones that bind to different ligands on the surface of fungi we have begun to examine new platforms for analysis of the “captured organisms”. In this regard we had previously done preliminary studies on attaching specific phage clones to self assembling fluorescent bead microarrays in collaboration with Dr. David Walt at Tufts University Department of Chemistry. Unfortunately it proved difficult to bind the phage particles to the beads in a reproducible and functionally oriented manner. More recently we have collaborated with Dr. Guilford Jones and colleagues at the Boston University Photonics Center to study the possible use of surface enhanced Raman Spectroscopy (SERS) for the detection and discrimination of microbial species.

Surface enhanced Raman spectroscopy (SERS) is an optical technique that uses the radiation scattered from an incident visible or near IR laser to generate detailed vibrational spectra. This technology is exquisitely suited to the requirements for rapid, sensitive and specific microbial identification. Studies in several laboratories, including recent data from our laboratories,

demonstrate that Raman spectroscopy can generate a detailed species and even strain-specific spectral fingerprint.

IMPACT AND IMPLICATIONS

For SERS spectroscopy, the microorganism is placed on a nanostructured metal (e.g. silver or gold) substrate. The vibrational spectrum derives from the molecules that are most proximal to the surface. While highly specific, fingerprint Raman signals from the illuminated sample are strongly enhanced by the interactions with the metal surface. It may be possible to further enhance the signal intensity by capturing the microorganism onto that surface with phage displaying high affinity peptides. SERS spectral acquisition time is routinely on the order of seconds. This method probes cellular constituents to a depth of less than 100 Å from the metal substrate. Consequently, SERS spectral information is essentially limited to the microbial cell surface components thus contributing to spectral simplification and greater species differentiation. As shown in Figure 3, using our novel aggregated gold particle covered SiO₂ substrates, we have observed strongly enhanced, reproducible SERS spectra of bacteria at the single cell level.

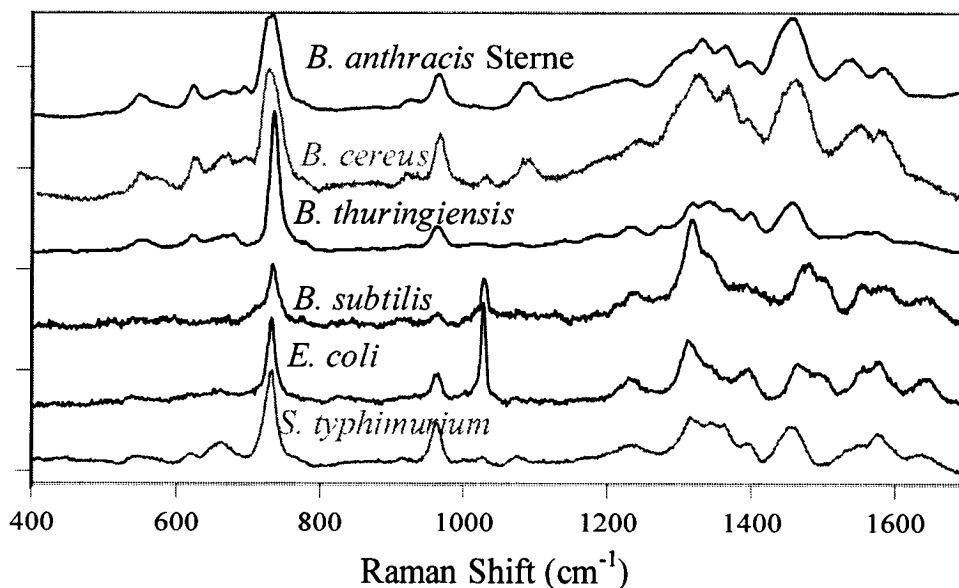


Fig. 3. SERS spectra of six bacterial species obtained on gold aggregate coated SiO₂ chips. Incident laser power 2 mW; data accumulation time 10 sec. Spectra are offset vertically for display purposes.

FUTURE WORK

During the next year we will focus our attention on the interface between the phage displayed peptide clones that we have obtained and the surface enhanced Raman spectroscopy system to determine if attaching either the cognate peptides or intact phage to the “chip” surface will further enhance the signal from bound microorganisms.

ADDITIONAL ACCOMPLISHMENTS

Using the techniques and concepts which underlie our NSBRI Smart Medical Systems project we have extended our research focus to another national priority “Biodefense”. We were awarded an NIH-NIAID grant (R21 AI53376 New Method for Detecting *Bacillus anthracis* spores) to investigate the possible utility of these methods to detect category A agents. Also, in

part based on our experience with these projects, we have submitted a grant application to NIH for using SERS in the detection of category A agents. Dr. Klempner is also the PI on the recently awarded National Biocontainment Laboratory to Boston University Medical Center.

PRINCIPAL INVESTIGATOR:	Lakshmi Putchu, Ph.D.
PROJECT TITLE:	Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration

A continuing challenge for space medical operations at NASA is the management of pathology associated with neurovestibular adaptation during space flight. A primary manifestation of this problem, particularly in the first few flight days of shuttle missions, is space motion sickness (SMS). The current treatment of choice for symptoms associated with SMS is promethazine (PMZ). Although oral tablets and rectal suppositories have been used during space flights, the intramuscular route appears to be most effective. On the other hand, intramuscular administration of drugs is an invasive procedure and PMZ causes irritation at the site of injection. A key research topic in the Smart Medical Systems area of the NSBRI 99-02 research announcement is development of novel therapeutic modalities for remote site medical operations such as space missions. In response to this initiative, the goal of the proposed research has been to develop an intranasal dosage formulation of PMZ that will provide crewmembers with a non-invasive means of self-administering SMS medications.

Accordingly, the following three aims were addressed:

- Develop a microencapsulated, pH balanced gel dosage formulation and a combination form with a corticosteroid for intranasal administration of PMZ
- Establish the release kinetics and shelf life of the optimized dosage forms, and
- Assess bioavailability, nasal mucosal irritability and toxicity of the selected dosage forms in rats.

Progress:

A microencapsulated formulation of promethazine hydrochloride (PMZ.) was developed for intranasal administration to treat space motion sickness. The formulation is designed to achieve zero order release with minimal “burst effect”, rapid initial release of the drug. An in vitro screening of different ointment and gel formulations containing absorption enhancers was conducted to assess uniformity of microcapsule delivery and release kinetics.



Fig. 1. Ethocel coated Stearine-27 (20% PMZ) Capsules

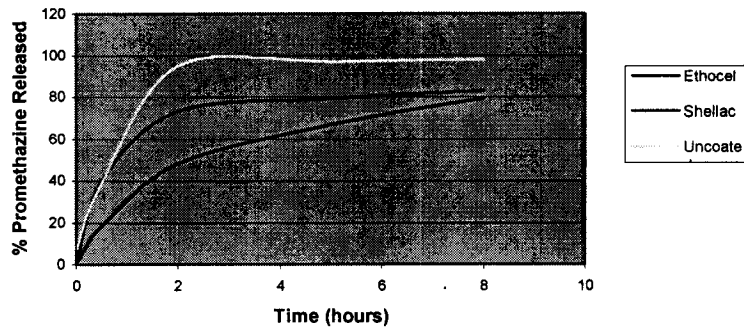
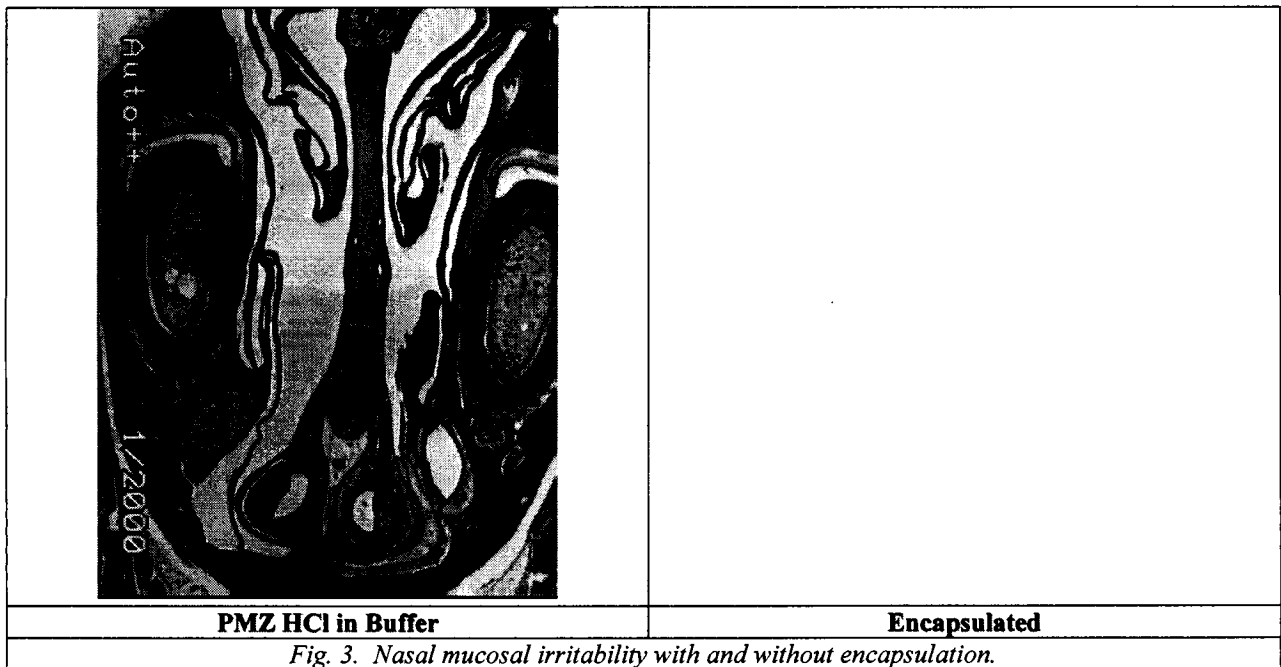


Fig. 2. Release Rate of Coated and Uncoated PMZ Stearine-27 Microspheres

Formulations selected from the in vitro evaluation were then examined for mucosal irritability and toxicity in an animal model. Results indicated that PMZ HCl is cytotoxic in a non-encapsulated delivery form at a concentration of 125 mg/ml both in vivo and in vitro. This cytotoxicity is independent of pH of the solution (both in vitro and in vivo). Encapsulation allows for controlled delivery at concentrations that do not elicit the cytotoxic response. Comparison of circulating drug levels after 30 min after intranasal administration to rats revealed that absorption from the selected microcapsule dosage forms was equivalent to that from normal saline, however, the nasal mucosal toxicity effects observed with the saline dosage form were absent with the microcapsule formulations.



Nasal Irritability and systemic concentration after Intranasal administration of the prototype dosage forms to rats

Treatment	Nasal Inflammation	Plasma PMZ at 30 min. **
Saline	None	None
PMZ in Saline	None	212
PMZ in Buffer	Extensive Inf.	266
PMZ Freebase *	Moderate Inf.	193
PMZ Capsule Prototype *	None	173
PEG/Glycofuralam	None	None

* In PEG/Gycofuralam ** (ng/ml)

Significant accomplishments:

- Invention disclosure application filed by SwRI
- Patent application process initiated with legal offices of NASA and SwRI
- Predictive model for drug delivery from encapsulation developed
- Presented results and current technology platform at national and international meetings
- Extension of technology application to other Operations-critical drugs initiated.

Implications of project findings for future research:

Microcapsule gel formulations are state-of-the-art dosage forms that will have a lot of potential in acute and chronic treatment modalities. Intranasal administration of drugs will offer an excellent alternative to conventional oral and parenteral routes, especially for pediatric and geriatric clinical care.

Presentations:

Putchu, L., Vasishtha, N., Persyn, J.T., Nino, J.A., McDonough, J.A., "Development of an Intranasal Controlled Release Formulation of Promethazine Hydrochloride," 29th Annual Meeting of the Controlled Release Society, Seoul, Korea, July 2002.

Putchu, L., Persyn, J.T., Nino, J.A., McDonough, J.A., "Determination of the Promethazine Hydrochloride Release Rate Dependence Upon Ethylcellulose Coating Technique and

Microcapsule Core Morphology in Preparation for Nasal Delivery,” American Association of Pharmaceutical Scientists Annual Meeting, Toronto, Canada, November 2002.

J.A. McDonough, E.J. Boland, J.T. Persyn, J.T. Nino, Z. Wang and L. Putcha, Development and Testing of a Noninvasive Intranasal Dosage Formulation of Promethazine Hydrochloride, Bioastronautics Conference, Galveston, Texas, January 2003.

PRINCIPAL INVESTIGATOR: Babs R. Soller, Ph.D.
PROJECT TITLE: Noninvasive Measurement of Blood and Tissue Chemistry

PURPOSE

The long term goal of this project is to create a noninvasive and lightweight system for measuring blood and tissue chemistry through the skin. The technology is based on Near Infrared Spectroscopy (NIRS) and is targeted to work on subjects of any ethnic origin. The system developed during this 3 year project will measure three metabolic parameters: muscle pH, muscle PO₂ (partial pressure of oxygen) and blood hematocrit. Such a system is planned to be part of smart medical systems used to 1) identify and treat traumatic injury, 2) assess fitness levels in on earth and in space and 3) to assist in the development of exercise countermeasures to diminish loss of muscle mass, strength and endurance during long duration space flight.

BACKGROUND

During Year 1 and 2 of this project we demonstrated the ability to accurately measure muscle pH and PO₂ on human subjects undergoing open heart surgery under cardiopulmonary bypass, a controllable model for metabolic changes in muscle under complex physiologic conditions. The results of this study were recently published (see below) and illustrate the capability to detect subtle metabolic changes over a wide range of body temperature and oxygen saturation levels. The next step is to develop robust calibration equations that are accurate for all subjects.

The development of robust calibration equations required improvements in hardware stability and techniques to assure repeatable sensor placement and invariance to biological variability. Hardware improvements are being addressed with additional support from the US Army Medical Research Command, which is interested in this sensor system for triage and treatment of battlefield casualties. The Army is also funding work to develop techniques to transfer the robust calibration equation (developed as part of this NSBRI project) to multiple instruments.

Additionally, during the last year we continued to explore NASA's interest in the metabolic sensor for assessment of fitness and development of exercise countermeasures. Donald Hagan, PhD, Exercise Lead, NASA JSC and Marco Cabrera, PhD, NSBRI investigator on the Nutrition and Fitness team continue to have interest in collaborating with us to develop the NIRS metabolic sensor for exercise applications. During this past year we began development of a modified sensor for use on leg muscle (vastus lateralis), as specified by Dr. Hagan.

RESULTS DURING REPORTED PROJECT YEAR

System Improvements for Robust Calibration

The system for our previous (published) work was constructed from a specially designed sensor, but off the shelf spectroscopic components. The new system (Figure 1) is not only smaller (8.625" W, 7.872" D, 4.625" H) but has specially designed hardware and software which provides exceptional stability, less than 1% signal variability over 12 hours.

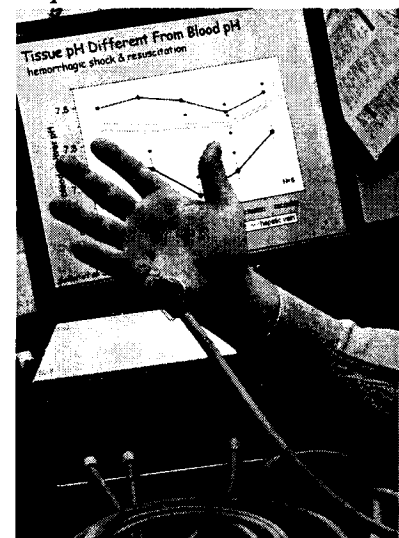


Fig. 1. New fiber optic cable and monitor for noninvasive metabolic measurements.

We also redesigned the fiber optic sensor to reduce its sensitivity to skin surface texture differences between subjects. This redesign also allowed us to investigate the effect of source - detector spacing on measurement accuracy and precision. This was first done on liquid, single layer phantom materials designed to optically resemble muscle or fat. The main goals were to determine the source-detector (S-D) spacing which has depth penetration at least as deep as the original probe but not so deep that light is returned from muscles or tendons near our target muscle. These results are presented in Figure 2. The phantom study showed S-D spacing between 3-8 mm will provide the optimum response for measurement on the palm of the hand (trauma application).

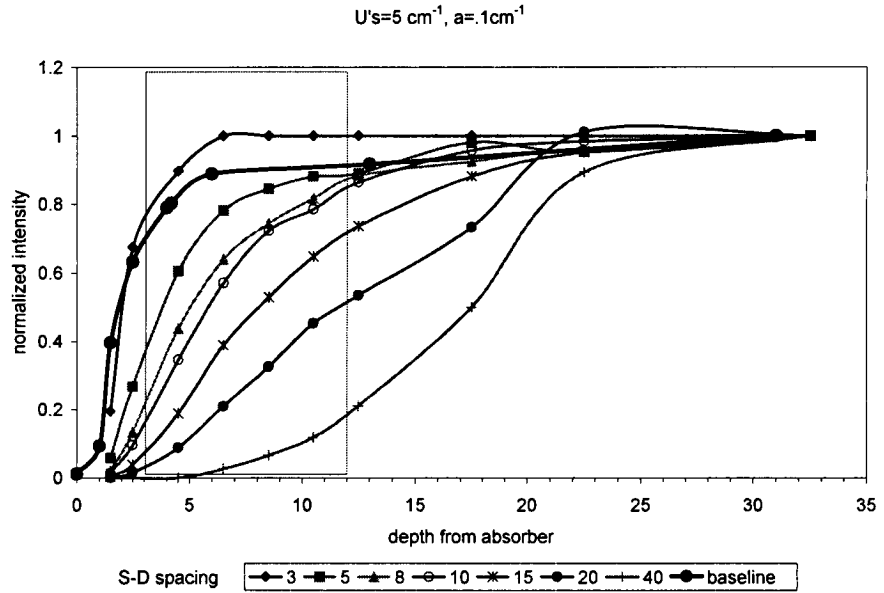


Fig 2. Source-detector spacing is compared to baseline (current) probe (black filled circles). μ'_s and μ_a were chosen to be values approximately equal to muscle scattering and absorbance at 760 and 840 nm. Blue box is where optimal response is required.

A special fiber optic probe with 2 rings of illuminating fibers spaced 4 mm and 8 mm from a single detection bundle was fabricated by our engineering and manufacturing partner, Luxtec Corporation. This special probe was then used to investigate which S-D spacing provided optimal precision and accuracy for our noninvasive metabolic measurements.

The probe was tested on 3 subjects (J01-J03) undergoing cardiac surgery and the results of the new probe at the 2 spacings were compared to our original results. Table 1 shows this data.

Table 1. Accuracy of muscle pH & PO₂ measurements for 4 mm and 8 mm S-D, compared to average of 18 patients measured with the original fiber optic probe

PO ₂	R ²	RMSEP	pH	R ²	RMSEP
original	0.71	5.7	original	0.84	0.021
4 mm			4 mm		
J01	0.92	3.6	J01	0.73	0.016
J02	0.87	2.7	J02	0.94	0.014
J03	0.92	6.0	J03	0.82	0.040
avg	0.90	4.1	avg	0.83	0.023
8mm			8mm		
J01	0.94	3.2	J01	0.84	0.012
J02	0.86	2.9	J02	0.95	0.014
J03	0.87	8.9	J03	0.83	0.040
avg	0.89	5.0	avg	0.87	0.020

The data in Table 1 show that either the 4 mm or the 8 mm S-D spacing will provide the same accuracy as that achieved with our original probe. This indicates that both probe geometries are successful in collecting light from the depth of the invasive sensor placement.

We also investigated the effect of probe size and probe location on placement precision. Ideally, 5 replicate placements of the probe on the hand should yield exactly the same spectrum. An adhesive target was designed to allow precise placement of the sensor relative to key anatomic features. The sensor is fixed to the palm using medical grade, double-sided adhesive (Figure 3). The placement precision of 5 replicate placements on difference size hands was assessed by calculating the standard deviation across these 5 repetitions. For all hand sizes and all locations the 4 mm S-D spacing resulted in superior placement precision (smaller standard deviation). The target muscle is the abductor digiti minimi. This muscle is trapezoid shaped and runs from the pinky to the wrist. On small hands scattered light from the tendon sheath adjacent to the muscle likely degrades precision. The smaller diameter probe, placed closer to the wrist ensured repeatable placement. For hand sizes ranging from extra small to extra large, the error resulting from placement was measured to be 0.007 absorption units, a variation of 3.3% when compared to the palm absorption .



Fig. 3 Fiber optic probe attached with double-sided adhesive, aligned to abductor digiti minimi muscle using white target.

The new fiber optic probe, placement system and stable monitor will be used to collect data from additional cardiac surgical patients to create robust calibration equation for muscle pH, PO₂ and blood hematocrit..

Leg Sensor Development

The fiber optic sensor and monitor described above must be modified for measurement of leg muscle during exercise. The experiments illustrated in Figure 2 also allowed us to get an initial look at depth penetration required for probing deep within the vastus lateralis muscle. Figure 2 shows that by using a source-detector spacing between 20 and 40 mm we will be able to penetrate to a depth of 20 - 25 mm within muscle tissue.

Initial development of the sensor and monitor for probing leg muscle will take place on phantoms designed to simulate a muscle layer covered by a variable thickness layer of fat. We developed procedures for creating realistic and stable solid phantoms. The base material for these phantoms is agar. The fat layer is created by mixing intralipid

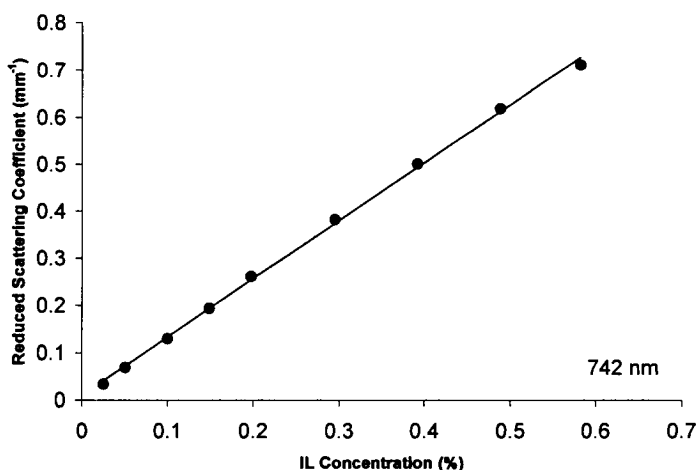


Fig 4. Derived relationship between intralipid concentration and reduced scattering coefficient.

into the agar. We determined the amount of intralipid required to vary the scattering coefficient to match that of a human fat layer. Figure 4 shows the derived relationship between intralipid concentration and reduced scattering coefficient.

The muscle layer is fabricated by mixing intralipid for scattering and diluted lysed blood for absorption in an agar matrix. The relationship between blood concentration and absorption coefficient was determined to allow careful control of the muscle layer optical parameters. This relationship is shown in Figure 5.

The blood containing agar phantoms were shown to have spectral stability for 9 days.

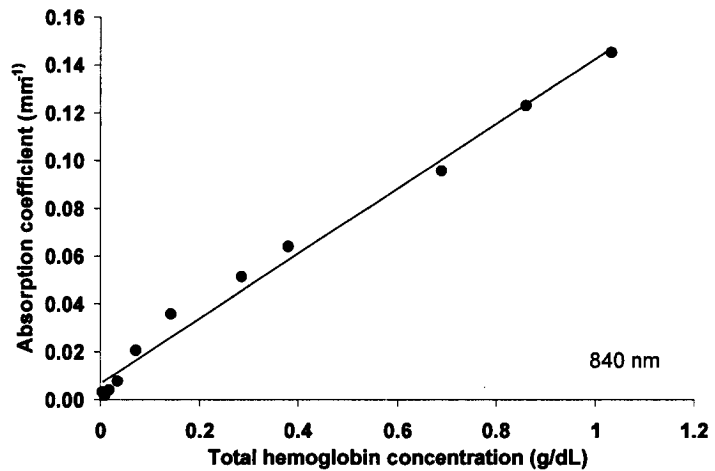


Fig 5. Derived relationship between hemoglobin concentration and absorption coefficient.

CONCLUSIONS AND IMPLICATIONS

- A miniaturized, portable, fiber optic spectrophotometer was designed and constructed. (Funded by the US Army Medical Research Command under grant DAMD17-03-1-0005 and constructed in collaboration with Luxtec Corporation). Novel circuitry and instrument design resulted in exceptional long term spectral stability. This spectrometer will have application for measurement of multiple analytes through the skin. The spectrometer itself is generic enough to perform fiber optic reflectance spectroscopy of any material.
- A new fiber optic cable and placement target were designed and fabricated to optimize placement precision on subjects with varying hand size and skin textures. This task was also completed with the assistance of Luxtec Corporation. The techniques developed here will enable us to meet measurement accuracy targets on subjects of varying anatomy (hand size), overcoming any gender related differences.
- Methods were developed to construct a stable, solid, 2-layer phantom which optically simulates muscle covered by a variable thickness fat layer. These phantoms will be used to optimize the fiber optic probe and optical instrumentation to acquire spectra from deep within the leg muscle.

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PRINCIPAL INVESTIGATOR:	Gary Strangman, Ph. D./Jeffrey Sutton, M.D. Ph.D.
PROJECT TITLE:	Near Infrared Brain Imaging for Space Medicine

Progress on our project over the past year has continued along two separate fronts: (1) the application of diffuse optical imaging (DOI) for brain imaging in a spaceflight-relevant context, and (2) the characterization of a demonstration system for automated, intelligent medical decision making based on continuous, multi-sensor input.

We have applied our simultaneous optical and fMRI recording technique to subjects that are performing a simulated docking task (spacecraft to satellite; SpaceDOCK) under both rested and sleep-deprived conditions. Functional MRI scanning during the performance of our space-relevant docking task (SpaceDOCK) indicates a broad network of focal brain regions involved in task performance, including primary visual and motor cortex, as well as prefrontal and lateral superior temporal areas. Most such regions are involved both under normal and sleep deprived conditions. However, under sleep deprivation, the spatial extent and magnitude of the activations (see Figure 1) increase significantly.

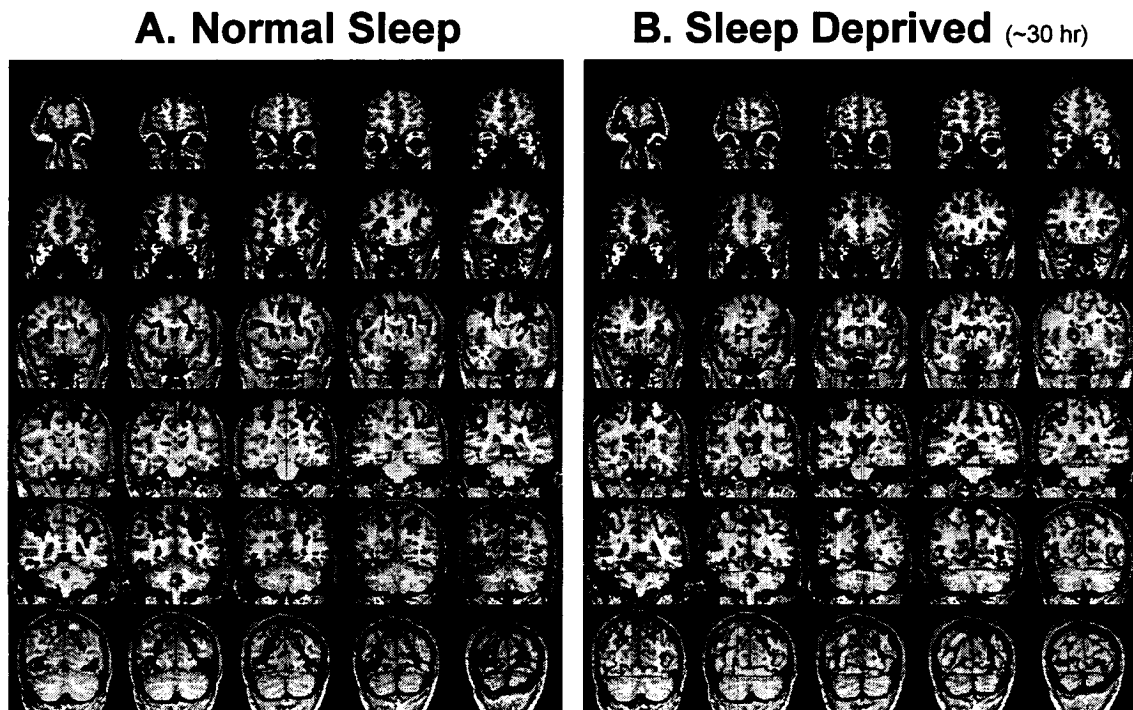


Figure 1: Significant differences in brain activation patterns as a function of sleep deprivation when performing SpaceDock (3D navigation/docking task developed as part of this project). A: BOLD fMRI brain activation map associated with task performance under normal sleep conditions. B: Identical map associated with sleep deprivation conditions. Significant differences are found in many locations, including in medial prefrontal cortex (i.e., beneath our optical probe; see Figure 2).

The DOI recordings are presently restricted to a smaller field of view on the brain relative to fMRI. However, within this field of view our findings indicate that DOI is at least as sensitive as (or perhaps even more so) than BOLD fMRI to hemodynamic changes consequent to neuronal activation. For example, in Figure 2B we show the activation signal modulation for both BOLD fMRI and the raw optical signals for the region circled in Figure 2A. Modulation

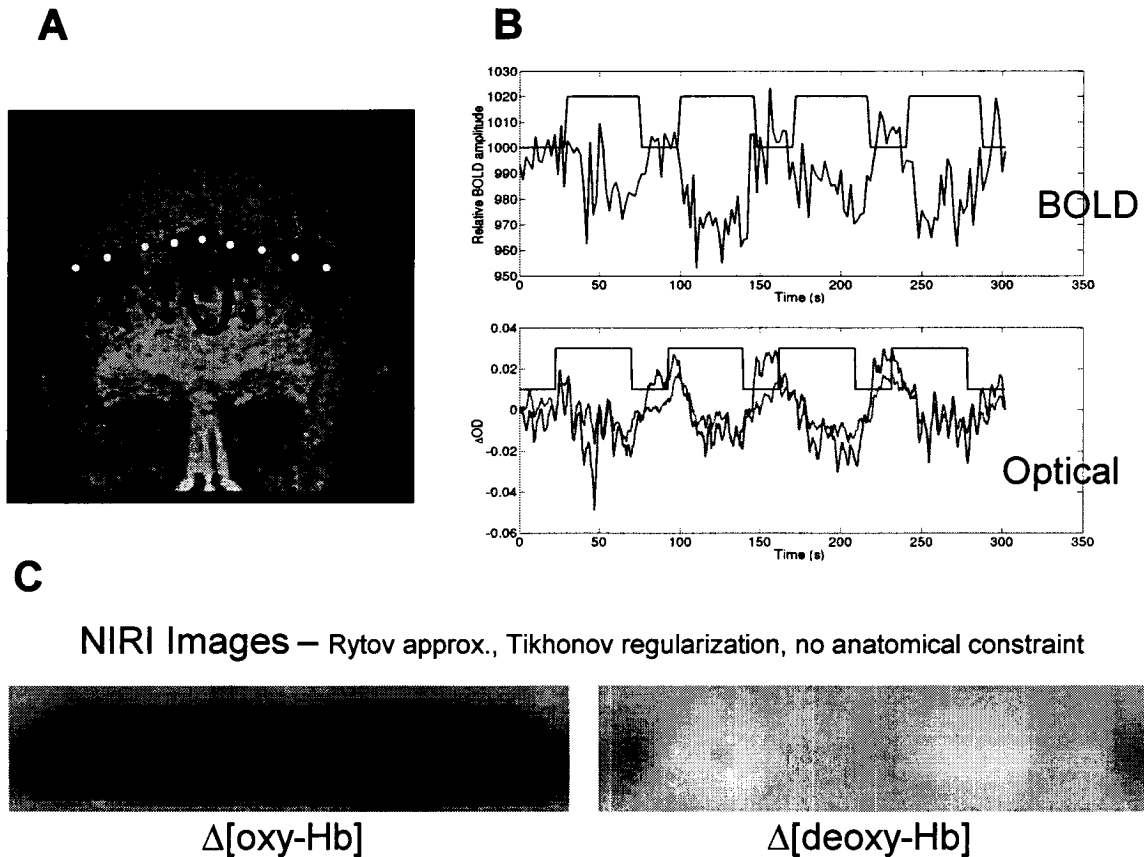


Figure 2: Diffuse optical imaging data, and comparison BOLD fMRI, averaged across 7 subjects. A: Rendered, anatomical MRI with co-localized positions of optical probes (red circle indicates the source-detector pair used for comparison). B: Comparison traces of BOLD fMRI (top) and raw optical signals (bottom; 785nm and 830nm), as compared to the task paradigm (blue square wave; low=rest, high=performing SpaceDock task). C: Reconstruction oxy- and deoxy-hemoglobin images from raw optical data, at time=200sec. Findings suggest that the decrease in BOLD signal is driven predominantly by a decrease in oxy-Hb concentrations, and not an increase in deoxy-Hb.

Moreover, Figure 2 provides uniquely interesting data in that simultaneous DOI and BOLD fMRI data were collected during a *decrease* in brain activation relative to the baseline task. Considerable controversy exists regarding the nature of such BOLD-based deactivations, and the simultaneous information about oxy-, deoxy- and total-hemoglobin provided by the simultaneous DOI recordings may help illuminate this controversy.

Finally, we have begun to characterize our Autonomous Intelligent Medical System. This system demonstrates the feasibility of using many sensors (from astronauts and their environment) to perform real-time evaluation and prediction of medical conditions and environmental changes. Figure 3 shows initial results of this characterization process, which captures most features of the demonstration system.

%Accuracy v %Noise v %Sparsity for Update 2 - Projection Strength 0.5

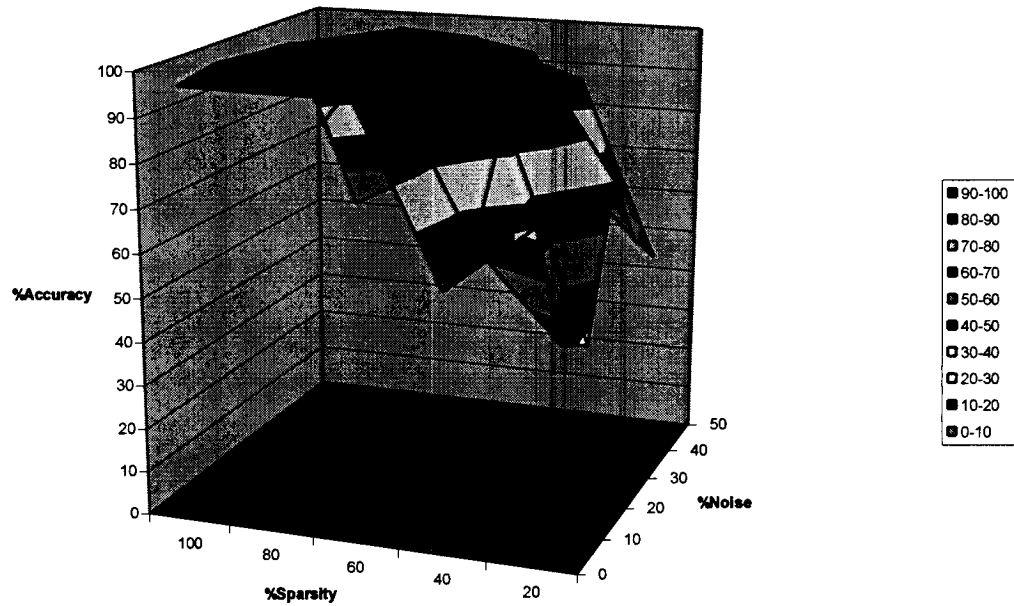


Figure 2: Evaluation of the AIMS system (accuracy of diagnosis across 10 different diagnoses with partially overlapping sensor signatures) as a function of sensor noise (results from 0-50% noise shown here), and sensor/input dropout (“sparsity”, where 100% corresponds to no dropout, and 0% corresponds to no data from any input sensors). The system performs very well across a broad region of this space, and only begins to fail when there is >50% dropout of sensory inputs. Update 2 corresponds to the situation where 50% of the final disease-state sensor values are presented to the network (i.e., a “50% presentation”).

To summarize, the system has been shown to be robust (that is, continues to provide accurate medical classifications) in the face of both sensor noise and dropout. This is perhaps the most critical feature for an autonomous medical system. We are further investigating the effects of the various model parameters and expect to have a manuscript describing the system and our characterization results submitted for peer review within the month.

Publications/Copyrights:

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PRINCIPAL INVESTIGATOR:	James Thomas, M.D.
PROJECT TITLE:	Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications

Background

A key goal of the Smart Medical Care System should be to optimize the use of diagnostic ultrasound in space and integrate it in an intelligent fashion with decision-making software. We have worked closely with NASA scientists, flight surgeons, and engineers to optimize research and diagnostic ultrasound aboard the International Space Station (ISS) and thus are well positioned to develop the necessary tools and techniques to integrate ultrasound into the Smart Medical Care System.

One of the principal limitations of ultrasound technology is its extreme dependence on the expertise of both the acquiring examiner and the interpreting physician. This is particularly true of two-dimensional ultrasound, where the examiner is required to obtain precisely oriented anatomical sections of the organ of interest. Three-dimensional (3D) ultrasound has the advantage of acquiring a large anatomic volume from a single ultrasonic window and thus may be less dependent upon the expertise of the examiner. Furthermore, this large volume may contain sufficient anatomic landmarks to allow unambiguous registration with previously obtained 3D data from either ultrasound or other modalities such as magnetic resonance imaging (MRI) or computed tomography (CT). One could thus envision a system by which whole organs or even the entire body would be imaged in 3D prior to launch, data that could be used to compare with subsequently obtained 3D datasets using in-flight ultrasonography.

The overall purpose of this grant is therefore to perform ground-based research, development, and validation aimed at optimizing diagnostic ultrasound in manned spaceflight, with the following unifying hypothesis: Serial 3D ultrasound examinations will enhance diagnostic capabilities in manned spaceflight. This hypothesis will be pursued with both hardware and software development, along with extensive validation studies.

The technical aspects of this program will be pursued with the following specific aims:

- Optimize acquisition methods for 3D sonography using reconstruction and real-time techniques.
 - Develop techniques for registering anatomical images from 2D and 3D ultrasound with those obtained from prior ultrasound examinations and from magnetic resonance and computed tomographic imaging, considered “gold standards” for non-invasive anatomical imaging.
 - Develop tools for abstracting, in an automated fashion, anatomic changes from serial 3D and 2D ultrasound studies.
 - Develop algorithms for the optimal compression of 3D ultrasound images.
 - Assess the ability of novice examiners to obtain 3D sonographic datasets after minimal training.

In addition to the practical application to manned spaceflight and the ISS, these systems each represent significant societal problems for which the incidence of disease is common enough to study within the time period of this grant. Furthermore, ultrasound is the standard diagnostic tool used in the management of patients with each of these conditions.

Progress

Clinical Significance of 3D Ultrasound Capabilities: To date we have performed over 2500 patient examinations with real-time 3D echocardiography, including exercise and intraoperative (epicardial) examinations, with quantitative validation in aneurysmal ventricles,¹ aortic regurgitation,² hypertrophic cardiomyopathy,³ mitral regurgitation,⁴ and dilated cardiomyopathy.⁵ We validated 3D color Doppler stroke volume⁶ as well as 3D reconstruction using a device identical to the ultrasound system on the ISS. Building on our experience with the Volumetrics RT3DUS, we have begun to use a much improved acquisition device (Sonos 7500) to obtain over 100 RT3D examinations in a wide variety of cardiac pathologies, including 28 acquisitions obtained epicardially in the operating room. Already, six abstract presentations and a journal article have resulted from this work including volume assessment validation, valvular assessment for surgical planning compared to standard 2D imaging, and surgical outcome results of myectomy in patients with hypertrophic cardiomyopathy⁷⁻¹³

Compression, Visualization, Segmentation, And Registration Techniques For 3D Ultrasound

Despite significant advances in ground-based and satellite data communications, digital transfer of data remains a formidable challenge. Optimistic NASA estimates indicate that the overall Deep Space Network (DSN) bandwidth will not exceed 400 kbps until the year 2004 and transfer rates not exceeding 1 Mbps until the year 2010.¹⁴ Therefore, assuming 100% error-free, 100% efficiency/availability, and complete dedication of the entire DSN bandwidth, transmission of a single cardiac cycle 3D volumetric dataset with an estimated size of 60 MB would take over 15 minutes. Clearly, it is easy to appreciate the need for high degrees of volume compression if diagnostic and potentially therapeutic ultrasound imaging is to be applied to future NASA deep space missions. Even the relatively low resolution Volumetrics device generates up to 60 MB of data per second and has proven cumbersome for visualization and quantification. To make this device applicable to space use, we validated the use of a 3D wavelet packet transform for 100:1 compression of 3D echo.¹⁵ We have shown the ability of maintaining a higher signal-to-noise ratio using a modified set partitioning in hierarchical trees algorithm (MSPIHT) over the conventional technique. Also, we have developed highly compact software for visualization,¹⁶ segmentation,^{17,18} and registration.^{19,20} These now can register pre- and post-exercise images and cross-modality data as described next.

Mutual Information-Based Registration of Ultrasound Volumes: We have shown that both rigid-body and affine misalignments can be recovered between volumetric ultrasound images using mutual information-based registration.¹⁹ In this approach, one of the two images (primary) is kept stationary and the other (secondary) is transformed iteratively until the optimal alignment, corresponding to the maximum of the mutual information (MI) function, is found. MI is an intensity-based measure, which conveys the amount of information that the primary contains about the secondary. MI is more effective than other intensity-based measures such as simple cross-correlation, since it is a statistical measure that works even if voxel intensities in the primary are nonlinearly related to those in the secondary. We search for the maximum of the MI

by searching for a set of geometric transformation parameters, which produce a 4 x 4 transformation matrix T . Upon convergence, the MI is maximized between the primary (A) and the transformed secondary (TB). T refers to rigid-body transformation if it incorporates rotation and translation only. The transformation is affine (nonrigid) if it includes scaling and shearing, as well.

Registration of Resting Cardiac Ultrasound and SPECT: We have also performed MI-based registration of gated Sestamibi SPECT and RT3D ultrasound images in a pilot study involving nine subjects.²¹ Since ultrasound collects more frames per cardiac cycle, 8 original SPECT frames were interpolated with the aid of associated electrocardiograms to match the available ultrasound frames. The average rating for success made by 5 experts was 3.58, with a 1 meaning poor registration and 5 meaning near perfect spatiotemporal registration. Of note, we detected virtually all clinically relevant abnormalities, such as a perfusion defect in the right coronary artery territory that matched with a wall motion abnormality in the inferior wall by 3DUS. Only 7 of 45 evaluations were scored below 3; these were primarily due to poor endocardial definition in ultrasound images – attributed to the quality of the Volumetrics 3DUS device rather than the registration procedure itself. Recently, the image quality of 3D ultrasound has improved significantly. We will further explore cross registration capabilities in Specific Aim #2.

Teaching Novice Examiners to Obtain 2D and 3D Data With Minimal Training: A major issue with diagnostic imaging in space is the limited training that astronauts are likely to have, given the other demands on their schedule. In an important recent study, 5 novices with only 4 hours of training were able to perform technically adequate 2D echocardiograms with remote coaching from an experienced sonographer. Quantitative parameters (e.g., LV mass, ejection fraction, mitral inflow) agreed with ($\leq \pm 10\%$) and correlated well with ($r > 0.85$) measurements by an expert.²² 3D studies of both heart and kidney are ongoing, but anecdotal observations from this work suggest that 3D imaging is even easier to learn, since precise orientation of the probe is not necessary, only obtaining images that include the structure of interest and are free of near-field and other artifacts.

Facilitate ISS Ultrasound Use and Optimize Digital Downlink Technology: After 4 years of work with JSC engineers during our first grant (NCC9-60), a Philips HDI-5000 ultrasound system was launched to the International Space Station in March, 2001.²³ Since then we have worked closely with Medical Operations physicians and engineers accomplishing several goals: optimizing the probe availability for this unit, testing digital file transfer from ISS to the ground, and developing live video feed capability for guiding the acquisition and real-time interpretation of data by a ground based expert. Digital echocardiography is now fully feasible on the ground²⁴ with over 250 studies being stored daily at the Cleveland Clinic (~3 terabytes annually). The American Society of Echocardiography has made all-digital storage and analysis a major policy goal of the organization, and the PI now chairs that effort.

Development of wireless technology to interface with a handheld digital ultrasound system: We continue to work with Kevin Montgomery of the Stanford Biocomputation Center/NASA Ames Research Center. Their approach has been to develop small, wearable sensors that register heart rate, respirations, temperature, etc.; then, digitize these signals, and transmit them using wireless protocols. The goal is to capitalize on the wireless development effort and the infrastructure

being developed at Ames and Stanford to allow full resolution (640x480x30 Hz) ultrasound to be made available in real-time on a web site with a latency of less than 2 seconds, short enough to allow realistic coaching and guidance of the acquisition by an off-site expert. To accomplish this, a wireless testbed within the Cleveland Clinic echocardiography laboratory is utilized to interface to the portable Sonoheart ultrasound device (Sonosite, Bothell, WA) via a PC with wireless capability using a video capture card. We have investigated echocardiographic exam review using Windows CE devices for their ability to view ultrasound data when connected using the 802.11 protocols.

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PRINCIPAL INVESTIGATOR:	James Thomas, M.D.
PROJECT TITLE:	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity

Background:

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations are generally well tolerated, but upon return to earth, astronauts often suffer from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities within the myocardium develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes.

Alone among imaging modalities such as radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Indeed, the Science Working Group (SWG) for the International Space Station (ISS) Human Research Facility (HRF) has recognized the primacy of ultrasound for medical diagnosis and physiology research, with plans to launch a specially modified commercial ultrasound instrument to the ISS in 2001. Echocardiography is similarly being used before and after shuttle flights and in a variety of bed-rest studies sponsored by NSBRI and NASA. Unfortunately, while ultrasound has the potential for high spatial and temporal resolution imaging of the heart, in the past it has been severely limited by operator inexperience and inconsistency in its subjective interpretation. Needed are new methodologies for assessing the load-independent function of the heart and consistent, objective quantification of a wide range of NASA echo studies, whether obtained on the ground, in flight, or in experimental models. We propose to provide such a facility while validating novel methods for the load independent assessment of myocardial function. Our central hypothesis is that microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties. A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS. However, within the scope of this grant, we propose several specific aims that will be critical to the ultimate comprehensive study of the cardiovascular system in space.

- Assessment of the effect of chronic volume and pressure unloading on ventricular myocardial properties
- Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic

intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates

- Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the *early detection* of myocardial dysfunction during prolonged space flight
- Development and distribution of stand-alone software and algorithms for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources
- Establishment of an Echocardiographic Core Facility for the NSBRI and NASA community, capable of applying standard and novel analysis techniques to data obtained from selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.
- If successfully implemented, these Aims will allow the cardiovascular sequelae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

Progress

The original grant submitted to the Cardiovascular Alterations Team from our group was partially funded, but we have endeavored to address as many of the Specific Aims in that original proposal as we could, consistent with the reduction in funding. Fortunately, productivity has remained quite high, and we feel very good about the progress that we have made in our current funding.

Impact of Volume/Pressure unloading on LV properties: As a model for LV mass regression in space, we have studied the dramatic LV regression that occurs when patients are relieved of their pressure or volume overload following aortic valve replacement for aortic stenosis or regurgitation. We had thus far enrolled sixteen patients with aortic stenosis and eight with aortic insufficiency, all of whom have undergone initial three-dimensional echocardiography but only a few of whom have undergone their late (twelve month) follow-ups. We have shown a fall in LV mass from $180 \pm 42 \text{ gm/m}^2$ at baseline to $149 \pm 34 \text{ gm/m}^2$ at six weeks post valve replacement ($P=0.03$), with a further fall at six months, though follow-up data are limited at this time period. Furthermore, we have looked at sixteen patients with hypertrophic cardiomyopathy undergoing septal myomectomy and have shown a fall from $250 \pm 80 \text{ gm/m}^2$ at baseline to $210 \pm 62 \text{ gm/m}^2$ at six months, far in excess of the amount of septal tissue resected, indicating the very significant degree of secondary hypertrophy that occurs in hypertrophic cardiomyopathy due to the left ventricular outflow tract obstruction. Our success with these two models of LV mass regression have encouraged us to continue work in the current proposal, but adding very significantly molecular studies to address the genetic and signaling pathways that may be at work here.

Noninvasive Echo Assessment of LV Function: We have continued rapid development of several new echocardiographic indices for quantifying systolic and diastolic function. We have demonstrated that myocardial systolic strain-rate is a noninvasive surrogate for the end-systolic elastance,¹ and have demonstrated regional variance in strain to be a powerful measure of success in biventricular pacing.² The stratified reference values have been obtained in 102 normals for tissue velocity, displacement, strain-rate, and strain for all four left ventricular walls at the basal, mid and apical levels, which has established normative data against which to compare changes in various pathologic states as well as in microgravity. We have assessed the

preload and inotropic dependency of tissue velocity in dogs and have shown that contrary to the popular notion, systolic tissue velocities in the normal heart are not independent of preload, this state only being observed in pathologic hearts or in hearts with delayed relaxation.³ Similar findings were confirmed by us in humans undergoing a microgravity mimicking bedrest in studies that we have collaborated on with Dr. Ben Levine in Dallas, Texas.⁴

We have completed much of our preliminary validation work for measuring the intraventricular pressure gradient (IVPG) from application of the Euler equation to color M-mode Doppler echocardiographic data of transmitral flow.⁵ We have used this to quantify the changes in diastolic suction following septal ablation in hypertrophic obstructive cardiomyopathy, a presentation that won the Young Investigator's Competition for the American College of Cardiology in 2002. We have also shown that the color M-mode propagation velocity is more independent of preload than tissue Doppler velocity, making it an attractive index to assess cardiovascular countermeasures in space.³

Exercise Assessment for Early Detection of Myocardial Dysfunction during Prolonged Spaceflight: We have conducted a number of clinical studies demonstrating the efficacy of exercise echocardiography in detecting subclinical disease. In a recent study, we studied 31 patients with heart failure and 15 normals before and after submaximal metabolic stress testing, applying the Euler equation to their color M-mode Doppler data to calculate IVPG. Although the resting IVPG was only weakly associated with the maximal exercise capacity ($\text{VO}_2 \text{ max}$), the increment in IVPG (2.6 ± 0.8 mmHg in normals vs. 1.1 ± 0.8 mmHg in patients, $P < 0.05$) was the best predictor of exercise capacity ($r = 0.80$, $p < 0.001$).⁶ We have also published a validation demonstrating the use of a simplified index of cardiac power as a way to monitor cardiac reserve, a very simple approach that may have important application in space.^{7,8}

Development of software for Quantitative Assessment of LV Function As part of our development work for Aims 2 and 3, we have developed stand-alone software for the quantification of IVPG from color M-mode data and strain data from 2D tissue Doppler data. These can be applied to DICOM formatted data from any echo machine, including the HDI-5000 on the ISS.

Assessment of Myocardial Mechanics using 2D Echocardiographic Strain measurement: In the past 3 years, our group and others have worked to develop echocardiographic measurements of LV strain. Currently, this is done by differentiating in space and integrating in time a tissue velocity map,⁹ an approach that has proven valuable in studying patients with coronary disease, cardiomyopathies, and diastolic dysfunction. However, this Doppler-derived strain is highly sensitive to signal noise and the angle of ultrasound interrogation and only provides a single strain component. Extraction of myocardial mechanics by "speckle tracking" of 2D echos was proposed almost 20 years ago, but accurate quantification of regional 2D strain has become feasible only recently with the advent of high-resolution, high-frame rate grey-scale images.¹⁰ A comprehensive assessment of cardiac motion and deformation can be achieved providing myocardial tissue velocities, strain rates and strains without any angle dependency. Our initial experimentation with this algorithm has been very encouraging regarding its utility and accuracy. Regional velocity and strain measurements have been compared in 80 individual segments from 5 subjects using standard tissue Doppler measurements and 2D speckle tracking. A strong

correlation ($r=0.79$) between these approaches has been observed, the discrepancy likely reflecting that the principle (maximum) strain vector can be obtained only in 2D, while the tissue Doppler approach is limited to the component along the ultrasound beam. A truer comparison is between 2D strain measurements by speckle tracking vs MRI. In 6 patients, the average circumferential strain by MRI tagging analysis was $-21.8\pm 4.3\%$ vs. $-21.0\pm 3.1\%$ by 2D speckle tracking. The average strain difference was only $0.7\pm 3.7\%$, indicating outstanding agreement.

Echocardiographic CORE facility for NSBRI and NASA:

We continue to be quite busy in our core analyses for the NSBRI funded bedrest studies in Boston (Richard Cohen: PI) as well as NASA funded work in Dallas (Ben Levine, PI). We are also collaborating with Dr. Lakshmi Putha (Johnson's Space Center), to quantify hepatic flow as well as to Drs. Lorell and Schneider concerning the measurement of cardiac strain in rodent models of microgravity. Through the Smart Medical Care Team, we are also working with Dr. Lawrence Crum to couple improved ultrasound diagnostics with his high intensity focused ultrasound (HIFU) method for cauterization and tumor ablation.

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**National Space Biomedical Research Institute
ANNUAL TEAM REPORT**

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**National Space Biomedical Research Institute
ANNUAL TEAM REPORT**

Technology Development Team

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I. ABSTRACT

The objective of the Technology Development Program of the National Space Biomedical Research Institute is to develop devices, instrument systems, and associated algorithms and software that lead to a better understanding of the barriers to long-duration space exploration and assist in the development of countermeasures to assure safe and productive space missions. The primary focus of the Technology Development Program is directed towards those technologies that support the ground-based and space-based activities of the other NSBRI Research Teams. The unique feature of this program is the opportunity to bring an integrated system engineering perspective to bear on the technological developments necessary to support basic research.

Eight instrument or device development projects are currently part of the NSBRI's Technology Development. Three of the projects are in their second three-year cycle and continue to demonstrate excellent progress in achieving their individual goals and objectives (operational instruments collecting data under realistic use conditions). Four of the projects are in the first three-year cycle (started February 2001) and all have made substantial progress consistent with their start dates and stated goals and objectives. All of the seven projects referred to above will be ending their current award cycle either by the end of calendar 2003 or in the first half of calendar 2004. These projects are identified below as numbers 1 through 7. The eighth project (number 8 below) did not start until July 2002 and is now in its second year of funding. To preclude unexpected technology issues and assure that the projects address needs established by the other Research Teams, rigorous reviews of both the continuing and new projects were conducted during the year. Each project team was encouraged to work closely with one or more of the other Research Teams that would benefit from their project's development. The eight Technology Development Team development projects directly support the technology needs of nine of the ten remaining NSBRI research teams. In several of the projects, prototype instruments and systems are already operating and have moved along the path to the establishment of definitive scientific results.

The eight Technology Development Team projects are:

1. Advanced, Multiple Projection, Dual Energy X-ray Absorptiometry (AMPDXA) Scanning System. A highly accurate precision measurement instrument for bone mineral density, bone structure, muscle loss, and determination of bone fracture risk.
2. Neutron Energy Spectrometer. A lightweight, portable instrument capable of accurately measuring the neutron energy spectrum of the energy range from 20 keV to 500 MeV.
3. Miniature Time-of-Flight Mass Spectrometer. A portable, highly accurate mass spectrometer system capable of identifying biomarkers in urine, blood, saliva, and breath samples.
4. Improved Bubble Detection System. A new system (based on ultrasound) to locate and monitor nitrogen bubbles in human tissue and blood as well as extra-vehicular activity.
5. Scanning Confocal Acoustic Diagnostic (SCAD) System. An ultrasound system designed to measure bone loss in space due to microgravity effects.
6. Heavy Ion Microbeam/Detector System. A system aimed at studying radiation effects at the cellular level in order to better understand human health in high radiation environments.
7. Dynamic Exercise Countermeasures Device (DECD). Uses jumping as a mode of exercise for astronauts as a direct countermeasure to microgravity-induced muscle and bone loss.
8. Space Qualifiable Magnetic Resonance Imaging (MRI) System. The MRI project goal is to develop an MRI system capable of flying in space to perform small animal studies.

II. INTRODUCTION

The Technology Development Program (Team) of the National Space Biomedical Research Institute (NSBRI) is chartered with developing technologies that will lead to a better understanding of the barriers to long-duration space exploration and assist in the development of countermeasures to assure safe and productive missions. The primary focus of the Technology Development Program is directed toward those technologies that support the ground-based and space-based research of the other NSBRI research teams and space life science research community at large. Accordingly, it creates systems and tools such as sensors, instruments, devices, and intelligent software. Requirements for these tools and technologies are predicated on the carefully developed needs of the other research teams. In particular, the Technology Development Team selects projects that: (1) support the investigation of the effects of spaceflight on human physiology and behavior; (2) apply this information toward the development of techniques, technologies, instruments, and countermeasures that will sustain humans during future long-duration space missions; and (3) benefit the quality of life and medical care on Earth.

Synergism is a key element of the program and the Technology Development Team strives to bring the engineering and biological science disciplines together in the identification and development of devices, instrumentation, and systems that address the fundamental research issues critical to the human exploration of space. A unique feature of the Technology Development Team's projects are their ability to bring an integrated systems engineering perspective (cross discipline) to bear on technology development as it supports the basic research. An important by-product of this integrated approach is the cross-education of the basic and applied science researchers in engineering and technology disciplines and the applied research and development engineers in biological and medical science.

Since such a myriad of potentially useful technologies, devices, and instruments could have significant impact on an astronaut's health and his ability to perform his mission, it is important for the Technology Development Team to identify the most important of NASA's risk factors for humans in space and then from these risk factors identify technology applications and devices that might produce significant risk countermeasures or aid human adaptation or health care in prolong flight environments.

The Critical Path Roadmap (CPR) provides the foundation needed by NASA to ensure that human spaceflight now and in the future is as safe, productive, and healthy as possible (within the mission constraints) regardless of the mission duration or destination. The CPR provides a framework for risk identification, risk prevention, and the need for viable countermeasures associated with humans in long-duration spaceflight. The Technology Development Team uses this roadmap as one means of prioritizing project selection. For example, bone loss in microgravity is considered one of the most serious risk factors (Type 1). Bone loss and the causal or associated muscle alteration in space are being addressed by two NSBRI research teams. The Technology Development Team has ongoing projects that directly support the efforts of both the bone and muscle teams.

Using the roadmap alone is not sufficient to identify all technology needs of the NSBRI research teams as well as the needs for human spaceflight. The Technology Development Team actively engages members from the other research teams, the medical science community, and NASA to assess additional technical requirements. Through various individual team leader and working

group interactions, the needs are identified, distilled, and then focused into a technology development program.

The Technology Development Team has the following goals for its program:

Goal 1: Identifying new technological advancements and developments that can have a major impact on space biomedical research and astronaut health.

Goal 2: Contribute to risk reduction in each CPR priority area by developing new medical instruments and devices for both ground- and space-based research and countermeasure development.

Goal 3: Exploit the developments and advances made by Technology Development Team projects to improve the quality of life and health care delivery on Earth.

Goal 4: Promote the transfer of NSBRI-developed technological advances to industry for the benefit of Earth-based medical care.

Goal 5: Integrate technology development needs across other NSBRI teams, medical science community, and NASA through service and communication to become recognized as an important service arm that helps these researchers develop needed tools and instrumentation.

III. TEAM STRUCTURE & DESIGN

The risks associated with long-term exposure to microgravity and a high radiation environment are numerous; they represent the basis for the research program pursued by the NSBRI. Most of the ongoing NSBRI research is vertically integrated within a specific thrust area. For instance, the research teams typically have a core research topic that is combined with several special topic areas to form a disciplined approach to addressing a number of related issues.

The Technology Development Program is implemented in a different manner. The funded projects are selected, among other reasons, for their ability to provide necessary and enabling technologies for the basic research areas. Thus, the thrust area is laterally integrated with the other research areas. Figure 1 is a diagram showing the interaction between the eight current Technology Development Team projects and the other ten NSBRI research teams. As can be seen in Figure 1, the current Technology Development Team projects support nine of these remaining ten research areas.

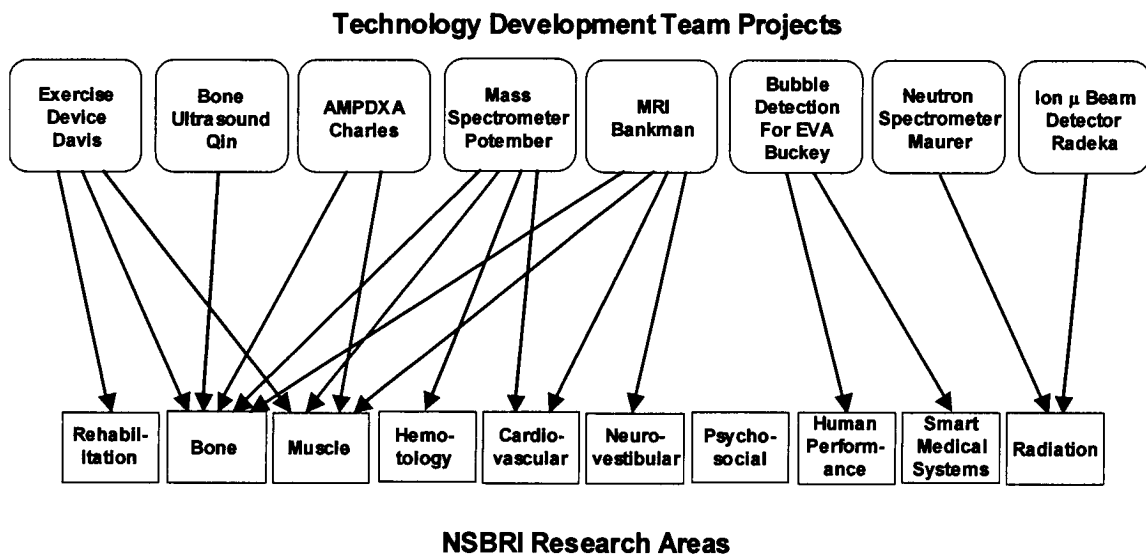


Figure 1. Mapping of current NSBRI Technology Development Team projects into the remaining ten NSBRI research areas.

The Technology Development Team generally focuses on projects that will deliver a specific product (e.g., sensor, instrument, etc.) in a specified period of time, typically one to three years. Of particular interest are projects that have strong technology transfer potential to industry so that the products of the development can be made available to support the research activities of other teams and achieve maximum societal benefit. Projects under the Technology Development Team umbrella are encouraged to interact with industry early in the development cycle. The NSBRI's Industrial Forum helps foster such interactions.

Proposals to the NSBRI Technology Development Program are expected to be of sufficient maturity (i.e., NASA Phase A (Conceptual Design)) so that: (1) the critical research issues can be readily identified, (2) the relevance of the technology development to the key research needs of the other NSBRI research teams and human spaceflight is evident, and (3) the technical approach

and development plan directly lead to a deliverable (instrument, sensor, countermeasure device, etc.) within a reasonable timeframe.

Currently, there are eight projects under the umbrella of the NSBRI Technology Development Team. As shown in Figure 1, the Technology Development Team projects address research needs and risk reductions in nine of the ten NSBRI basic research areas.

Project 1

PI/Project Title: Harry K. Charles, Jr., Ph.D.
Advanced Multiple Projection Dual Energy X-ray Absorptiometry
(AMPDXA) System

Need(s) Addressed: Provide accurate measurement of bone and muscle loss both in space and on Earth. Measure the location of the bone loss and assess the integrity of the bone structure.

Countermeasures Target: Provide highly accurate bone mass loss and structural information so that appropriate countermeasures can be developed, applied, and monitored.

Project Description: The purpose of the Advanced Multiple Projection Dual Energy X-ray Absorptiometry (AMPDXA) Scanning System project is to design, build, and test a precision scanning system for monitoring the deleterious effects of weightlessness on the human musculoskeletal system during prolonged spaceflight. The instrument uses dual energy X-ray absorptiometry (DXA) principles and is designed to measure bone mineral density (BMD), decompose soft tissue into fat and muscle, and derive structural properties (cross-sections, moments of inertia). Such data permits assessment of microgravity effects on bone and muscle and the associated fracture risk upon returning to planetary gravity levels. Multiple projections, coupled with axial translation, provide three-dimensional geometric properties suitable for accurate structural analysis. This structural analysis, coupled with bone models and estimated loads, defines the fracture risk. The scanner will be designed to minimize volume and mass (46 kg goal), while maintaining the required mechanical stability for high-precision measurement. The AMPDXA will be able to detect 1% changes in bone mass and geometry and 5% changes in muscle mass.

Two instruments (the Laboratory Test Bed (LTB) and a Human Test Bed (HTB)) have been constructed to date. The LTB has been used to develop source and detector parameters and to test human bone segments. The LTB has been fully operational for the last five years. It has allowed the AMPDXA project to develop sources, detectors, and software algorithms necessary for the high-precision detection of BMD and bone structure. In this current period, the LTB has allowed the refinement of our BMD and structure extraction algorithms as well as making continued progress on the high-resolution separation of soft tissue from bone. The HTB has been designed to conduct human patient testing. The HTB is operational and testing has begun.

Project 2

PI/Project Title: Richard H. Maurer, Ph.D.
Neutron Energy Spectrometer

Need(s) Addressed: Monitor the neutron radiation environment inside spacecraft, large space habitats, and on planetary surfaces.

Countermeasure Target: Provide highly accurate neutron radiation monitoring so that appropriate countermeasures can be developed, applied, and monitored.

Project Description: A Neutron Energy Spectrometer (NES) is being developed to monitor the flight radiation environment on the International Space Station (ISS), an interplanetary transport vehicle, or on a planetary surface. Detector types were selected for the complete neutron energy range and experimentally validated the concept for the low- and high-energy intervals. The effectiveness of our charged particle discrimination system was demonstrated. Data analysis and modeling efforts have verified the experimental results to date and the procedure for deconvolving deposited energy spectra into incident neutron energy spectra. The engineering prototype instrument has been successfully flown on a NASA aircraft and a high altitude balloon, demonstrating the robustness and operational capability of our design.

Project 3

PI/Project Title: Richard S. Potember, Ph.D.
Miniature Time-of-Flight Mass Spectrometer

Need(s) Addressed: Develop a miniaturized instrument that can quantitatively measure critical biomarkers from breath, body fluids, products of infection, etc.

Countermeasure Target: Provide a highly accurate measure of human biomarkers associated with many of the deleterious effects and conditions caused by microgravity and prolonged spaceflight so that appropriate countermeasures can be developed, applied, and monitored.

Project Description: The long-term objective of the Miniature Time-of-Flight Mass Spectrometer (TOFMS) project is to design, build, and launch a flight-qualified TOFMS for use on space platforms such as the Shuttle, ISS, or a planetary mission. The TOFMS can identify and quantitatively measure critical biomarkers associated with the deleterious effects of microgravity and long-duration spaceflight. The biomarkers can be determined from the analysis of breath, body fluids, products of infection, and, perhaps, DNA repair products and DNA mutations. As currently configured, the system appears to be of particular value to both the Bone and Muscle Teams, but biomarkers important to several other research teams can also be obtained. The TOFMS system being developed is small (less than 1 cubic foot), lightweight (less than 5 kg), low power (less than 50 W), and rugged. This NSBRI-sponsored TOFMS is building upon technology developed for DARPA to analyze chemical and biological weapons, while being optimized for astronaut use and the identification and quantification of biomarkers.

Many of the biomarker identification procedures are complex, requiring special protocols and associated laboratory equipment. To carry the equipment and chemical supplies required to monitor the health of an astronaut would be weight prohibitive, would necessitate specialized training, and would require a significant fraction of the astronaut's time. The TOFMS provides a small, efficient, broadband diagnostic instrument that can rapidly identify biomarkers important for successful human space exploration.

Project 4

PI/Project Title: Jay C. Buckey, Jr., M.D.
Improved Bubble Detection for Extra-Vehicular Activity

Need(s) Addressed: Careful monitoring and understanding of the bubble nucleation process associated with decompression sickness is required to reduce astronaut risks associated with extra-vehicular activity.

Countermeasure Target: Provide an understanding of blood bubble nucleation and growth so that effective countermeasures can be developed, applied, and monitored.

Project Description: The Improved Bubble Detection for Extra-Vehicular Activity (EVA) project goal is to improve current bubble detection methods. The assembly of the ISS requires extensive and unprecedented extra-vehicular activity. Because spacesuits operate at low internal pressures, the astronauts are highly susceptible to decompression sickness (DCS) (gas bubbles in the blood). A range of pre-breathe strategies, as well as suit gas mixtures and pressures, are employed to mitigate the risk. During EVA activities aboard the Shuttle and the International Space Station, in-suit Doppler bubble monitoring will be provided to detect conditions that increase DCS risk. Doppler bubble detection, while effective, has three primary limitations: (1) it is motion sensitive; (2) it detects only moving bubbles; and (3) it does not detect bubbles with diameters less than 80 μm .

The Improved Bubble Detection for EVA project will exploit two transcutaneous ultrasonic bubble detection and sizing instruments under development by NASA. These instruments utilize bubble resonance (not Doppler) techniques, thus allowing the instruments to measure stationary bubbles as well as bubbles of smaller size. One instrument is optimized for intravascular bubble detection in the size range of 30 to 200 μm . The other monitors extravascular bubbles in the 1- to 10- μm -size range.

Project 5

PI/Project Title: Yi-Xian Qin, Ph.D.
Scanning Confocal Acoustic Diagnostic (SCAD) System

Need(s) Addressed: Measurement of bone loss in space so that appropriate countermeasures can be developed, applied, and monitored.

Countermeasure Target: Provide measurement of bone material properties and relate to countermeasure development and processing.

Program Description: The Scanning Confocal Acoustic Diagnostic (SCAD) System project is focused on the measurement of bone loss in space. On Earth, early diagnosis and proper treatment of progressive bone loss (and/or poor bone quality) can dramatically reduce the risk of bone fracture. Ultrasound systems have the potential for determining the material properties of bone in a safe, repeatable, and highly accurate manner. Limitations in the performance of current ultrasound systems restrict their application to first-order screening, rather than the clinical standard upon which osteoporotic diagnosis and treatment regimens are based.

The SCAD is usable not only for ground-based determination of bone's physical properties; but, because of its low weight and size, it is also suitable for monitoring subtle changes in bone density and strength during extended spaceflight. The SCAD project is divided into four basic parts: (1) development of the SCAD system hardware, (2) correlation of SCAD-determined sound velocity and attenuation measurement with micro-CT bone BMD and structure, (3) prediction of the risk of trabecular bone failure associated with osteoporosis in the animal model, and (4) correlation of SCAD-derived BMD and structural modules with DXA measurements.

Project 6

PI/Project Title: Veljko Radeka, Ph.D.
Heavy Ion Microbeam and Micron Resolution Detector

Need(s) Addressed: The micron resolution detector, together with the microbeam, will allow the localized position of an ion impact within a cell to be determined. This is an enabling technique for radiobiology studies.

Countermeasure Targets: Understanding of the effects of radiation damage within the cell so effective countermeasures can be developed.

Program Description: The Heavy Ion Microbeam and Micron Resolution Detector System is aimed at studying radiation effects at the cellular level. Using microbeam irradiation facilities, it is now possible to place discrete numbers of particles in defined cellular and extracellular locations. Such facilities permit heavy-ion radiobiologists to explore the impact of signal transduction between cellular compartments as well as issues related to intercellular communication at low limiting fluences where not all cells in a population have been traversed. A high-energy, heavy-ion microbeam will allow an important unanswered question to be addressed, i.e., whether neurons that survive transversal by high-energy heavy ion (HZE) particles develop changes as a late consequence of the damage they incurred. These low-fluence studies will increase the understanding of the consequences of exposure to high, linear energy transfer (LET) radiation, such as encountered in the space radiation environment. (See the NES project above.)

The purpose of the Heavy Ion Microbeam and Micron Resolution Detector project is to allow such radiation studies as described above to take place by developing the following tools: (1) a microbeam (diameter 10 μm) of heavy ions (e.g., iron) at energies higher than existing ion microprobes (3 GeV/nucleon), and (2) an electronic position-sensitive detector for heavy ions with a position resolution better than 1 μm . Interactions between the Heavy Ion Microbeam and Micron Resolution Detector project and the Radiation Team have taken place.

Project 7

PI/Project Title: Brian L. Davis, Ph.D.
Dynamic Exercise Countermeasure Device (DECD)

Need(s) Addressed: Demonstrate that proper in-flight exercise can counter the microgravity-induced bone and muscle loss.

Countermeasure Target: Develop a direct countermeasure to bone and muscle loss in space.

Program Description: The Dynamic Exercise Countermeasures Device (DECD) is aimed at developing a countermeasure to bone and muscle loss in space. Bone demineralization (bone mass loss) is a well-documented physiologic effect of long-duration spaceflight and microgravity. Animal experiments on Earth have clearly indicated that: (1) certain bone strains and strain rates stimulate bone deposition, and (2) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column. Such studies have also indicated that a relatively small number of appropriate weight-loading cycles may be sufficient to stimulate bone deposition. Based on prior research with weight-loading experiments upon the foot, a dynamic exercise countermeasure device that utilizes jumping as the mode of exercise for the astronauts is under development. The DECD project is divided into three phases: (1) develop a lightweight, vibration-isolated exercise device, suitable for use on the ISS, that will permit dynamic jumping exercise within microgravity; (2) perform system testing using zero-gravity simulation; and (3) verify DECD efficacy in true microgravity through KC-135 experiments.

Project 8

PI/Project Title: Isaac Bankman, Ph.D.
Space Qualifiable Magnetic Resonance Imaging (MRI) System

Need(s) Addressed: MRI needed in space for animal studies and peripheral (limb) measurements on humans.

Countermeasure Target: Provide highly accurate bone and soft tissue measurements to verify countermeasures in space-based animal studies.

Program Description: The goal of the Space Qualifiable Magnetic Resonance Imaging (MRI) System is to develop a proof-of-concept engineering model of a space-qualified MRI system for small animals studies with a mass of less than 150 kg and low average power (<1 kW quiescent and <1.2 kW when scanning). An on-board processor or personal computer can be adapted to display the collected information. MRIs provide high-resolution, high-quality anatomical information without ionizing radiation, so they can be safely and repeatably used to track changes without deleterious effects.

As a result, the study of physiological alterations in space and the development, verification, and maintenance of countermeasures will be significantly enhanced. Mice and small rat models are useful surrogates to carry out in-orbit physiological studies. In-flight MR imaging of these animals will be of particular benefit to countermeasure development by several of the NSBRI research teams.

IV. TEAM ACCOMPLISHMENTS

Project Accomplishments

The Technology Development Team supports the needs of the other NSBRI teams and NASA. Through close communications with these groups, this team develops devices, instruments, and systems to improve research techniques and medical care on the ground and in space. Projects of the Technology Development Team focus on designing lightweight, compact research tools and on developing simple, minimally invasive and non-invasive sampling and measurement methods. Currently, there are eight active projects being pursued by the NSBRI Technology Development Team: three projects continuing from the first research cycle (1998-2000) and four new projects that were nominally started in February 2001. Some projects had slightly later starts due to funding transfer issues. The eighth project (Space Qualifiable MRI) began in July 2002. Several projects have also operated with reduced funding due to NASA funding shortfalls for the NSBRI. Research program accomplishments during the last 12 months are reported below.

Project 1: Advanced, Multiple Projection, Dual Energy X-ray Absorptiometry (AMPDX)
Scanner System
PI: H. K. Charles, Jr.

This project used advanced sensor and detector design and fabrication techniques to develop a compact, storable, low mass, and low powered dual energy X-ray absorptiometry (AMPDXA) Scanning System that is capable of determining bone mineral density, bone cross sectional area, and bone moments of inertia at any body site.

This project supports the explicit needs of both the Bone Demineralization/Calcium Metabolism Research Team and the Muscle Alterations and Atrophy Team. The prototype system is capable of real-time monitoring of bone and potentially muscle loss at extremely high precision. Since the resultant measurements are patient specific, the system is useful for monitoring the effectiveness of countermeasures as well as determining the risk of fracture of individual astronauts under deployment scenarios. On Earth, the system is a natural adjunct to research on the effects of aging and disuse on bone integrity along with routine screening for osteoporosis and monitoring for efficacy of osteoporosis therapy.

The AMPDXA project has had several notable accomplishments during the last 12 months. These accomplishments were focused in the following areas: (1) Laboratory Test Bed (LTB), (2) Human Test Bed (HTB) for ground-based human testing, (3) Commercialization of the AMPDXA, and (4) prototype design for space applications.

Accomplishments:

- Laboratory Test Bed

The LTB has been fully operational for the last five years. It has allowed the AMPDXA project to develop sources, detectors, and software algorithms necessary for the high-precision detection of BMD and bone structure. In this current period, the Laboratory Test Bed has allowed the refinement of our BMD and structure extraction algorithms as well as making continued progress on the high-resolution separation of soft tissue from bone. Multiple-projection analysis enables the user to evaluate bone structural properties (e.g., bending strength) independent of subject

position and orientation. Analysis of LTB images and ancillary data has demonstrated an average coefficient of variation in the maximum and minimum moments of inertial (I_{\max} and I_{\min}) on the order of 1% for a three-projection estimate. Experiments with more than three projections have indicated even further reductions are possible. Similarly, both the LTB and the HTB (described below) have been utilized to check system accuracy and repeatability. Both of which have exceeded expectations.

Multiple-projection imaging has led to the ability to generate three-dimensional reconstructions of the imaged bones. Future progress has been made on relating the structural information extracted from the weight-bearing bones by the AMPDXA to the mechanical properties and ultimately the risk of fracture. Since the AMPDXA stores and analyses patient-specific mechanical as well as BMD information on each patient, the risk assessment for each individual will be unique and not based on population means as is done today with conventional DXA systems. Preliminary progress has also been made on the extraction of standard radiographic images using the AMPDXA.

- Human Test Bed (HTB)

The HTB is capable of translation and rotation of the image plane, unlike commercial DXA systems. The objective of this system, built on the chassis of a used CT scanner to save time and cost, is to provide three-dimensional bone structural information as well as directly determine magnification. During the current period, the machine control software has been refined along with the development of a complete patient imaging protocol. Human images have been taken. Last year, the AMPDXA was moved to modern testing quarters outside of APL's secure compound to an adjacent industrial park with other APL activities. This move has facilitated human testing trials with the HTB. During the year, the software utilized to control the AMPDXA HTB operation was improved and brought under configuration control. This has allowed the HTB to operate in a more robust and repeatable manner.

- Commercialization of the AMPDXA

The commercialization process for the AMPDXA is still progressing. Several venture capitalists are considering investing in APL's spin off company. Also, one or two licensing opportunities are being pursued. A new, low-cost commercial unit design called the Ground Clinic System (GCS) has been designed. This unit, because of some very clever system design, has achieved multiple projection capabilities without the need for gantry rotation.

- Space Prototype System

Launch weight and spacecraft payload size limitations are serious factors associated with the viability of a piece of flight hardware. Without these constraints, commercial DXA systems are primarily designed for subject comfort and convenience. The project team has pressed hard to achieve exceptional system performance while also accommodating ease of use, minimum size, and a significant reduction in weight. The HTB was fabricated with existing components to meet the budgetary constraints, but a design exists of a system with a mass estimate of 86 kg, which is notably lower than the originally projected weight of 100 kg. In the 1-3 year timeframe, it is expected that advancements in x-ray tube technology will further reduce the weight to approximately 60 kg. In about 3-5 years, all of the component technologies (i.e., x-ray tube, detector, power supply, electronics) will have matured to the point where a target weight of

approximately 46 kg will be achievable. The recently invented high-efficiency source should help in this regard.

As mentioned above under commercialization, a configuration for the AMPDXA has been conceived that offers design simplicity resulting in lower development and manufacturing costs. This unit would also be suitable as a starting point configuration for a space mission.

Project 2: Portable Neutron Energy Spectrometer
PI: R. H. Maurer, Ph.D.

Galactic and solar cosmic rays are inordinately effective at producing secondary neutrons when they encounter spacecraft or habitat material. These neutrons can cause cellular and DNA damage to those exposed. The neutron component of radiation in a space structure is estimated to be between 30 to 60 percent of the total radiation environment when outside the Earth's magnetic field. To be able to measure the neutron spectrum, a portable brief case size, real-time neutron spectrometer prototype with a mass of less than 10 kg has been developed to support the research of the Radiation/DNA Effects Research Team. It can be used to characterize the environment for the development of a countermeasure and also can be used as a real-time monitor to control the application of countermeasures. The instrument measures neutrons in the range from 10 KeV to 500MeV with at least 10 percent energy resolution and count the number of neutrons below 10 KeV. This portable instrument incorporates the latest advances in energetic particle detection technology, including energy loss and total energy measurement, while building on the successful charged particle instruments built by JHU/APL for NASA/GSFC and NASA/JPL for many previous near-Earth and planetary missions. As the neutron energy spectrum is measured, an incorporated alarm will warn astronauts when a safe threshold is exceeded. The device, because of its small size, can be ported within a space vehicle or on a planetary surface to map the local neutron environment. This project addresses an explicit need of the Radiation Effects/DNA Damage and Repair Team.

Accomplishments:

- Modeling and Analysis

Modeling of the response of the high-energy channel from detailed cross-sections of the basic neutron-silicon interactions was undertaken using state-of-the-art computer codes. The purposes for developing the models were: to assess the accuracy of these codes for neutron-silicon interactions; to use these codes to understand the results of the energy deposition measurements; to determine whether these codes can be used to calculate the effects of packaging and instrument surroundings on the incident neutron spectrum; and, to assess the ability of the codes to supplement the experimental determination of the instrument response function. The current model using GEANT4 (a code library widely used in high energy detector design and simulations produced at CERN) reproduces the energy deposition spectra measured at RARAF reasonably well, although discrepancies for the highest energy depositions remain to be resolved. One interesting result of this model is a discrepancy at low energies indicating that gamma production during exposure may not have adequately been accounted for by measuring the background spectrum when the beam is off. In further work, a deconvolution technique that calculates a most probably incident neutron energy spectrum from the deposited energy spectra measures by the 5-mm thick silicon detector has been verified.

- High-Altitude Testing

The prototype neutron made several flights aboard F15 and F18 aircraft during the latter part of FY01. These flights were nominally at altitudes between 40,000 and 45,000 feet. Three balloon flights occurred during the last year reaching altitudes in excess of 85,000 feet. At 85,000-90,000 feet, the remaining atmosphere of nominally 23 g/cm² creates a high-energy neutron environment similar to that on the surface of Mars (the target Earth nuclei are nitrogen and oxygen, instead of the carbon and oxygen nuclei on Mars). The neutron environment at 23 g/cm² atmospheric depth is also equivalent to that experienced in a thick International Space Station module. The balloon flight used both a silicon solid-state detector and a Bicron 454 scintillator to monitor the neutron energy spectrum between 1 and 600 MeV.

- Materials Analysis and Testing

The proposed research, "Development of a Neutron Spectrometer to Assess Biological Radiation Damage Behind Spacecraft Materials," was selected for funding for the period May 2000 through November 2003. The primary responsibility under this grant is to support the Lawrence Berkeley Laboratory (LBL) personnel in the evaluation of spacecraft structural and shielding materials by supplying a version of the neutron spectrometer suitable for accelerator tests. The first experiments were conducted in the first quarter of 2001 at Brookhaven National Laboratory (BNL). In addition to work at BNL, studies involving 200 MeV protons at IUCF (November 2002), 500 MeV protons at TRIUMF (September 2003), and 500 MeV nucleon heavy ions at BAF (October 2003) were conducted. These experiments collide high-energy heavy ion beams with standard and novel spacecraft materials and the spectrometer measures the neutron energy spectrum produced as a result of these collisions. A secondary responsibility for this grant is to continue development of the modeling effort in a manner that is useful for materials science experiments as well as for assessment of astronaut biological radiation risk. The NES project team's work on shielding materials shows that polyethylene is an effective neutron shield. The team has found that for fast and high-energy neutrons, four inches (10 cm) of polyethylene is necessary to make a significant reduction in the neutron flux. For the fast (10-20 MeV) neutrons, a reduction of a factor of 3 was indicated while for the high-energy neutrons (20-600 MeV), a factor of 4.5 was determined.

Project 3: Miniature Time-of-Flight Mass Spectrometer
PI: R. S. Potember, Ph.D.

A high-resolution miniature TOFMS, already under development for other purposes, has been adapted for space flight. This instrument has the potential to identify and quantify a wide variety of biomarkers to support biomedical research and medical care. It is a rugged device that will unambiguously identify samples containing many compounds and be less than one cubic foot in size, weigh less than 5 kg, and require less than 50 W of power. Its applications include: analysis of breath, body fluids, products of infection, and perhaps DNA repair products and DNA mutations. Identification of compounds with mass ranges from under 100 to more than 10,000 amu has been demonstrated. While the instrument has a wide range of usage, the initial focus of the project is on the analysis of a variety of biological compounds in fluids although the instrument can be adapted to handle samples of various types. As currently configured, this instrument is of special value to the Bone Demineralization/Calcium Metabolism Team and the Muscle Alterations and Atrophy Research Team as well as being useful for gathering data on a variety of other experiments for the other Research Teams.

Accomplishments:

- Prototype Hardware

A major objective of this project was the design and development of a mass spectrometer *system architecture* that can be utilized for diagnostics based on complex, non-volatile biomarkers species. An orthogonal extraction time-of-flight mass spectrometer (TOFMS) analyzer, incorporating a dual matrix-assisted laser desorption/ionization (MALDI) and electron ionization (EI) source, was successfully completed and demonstrated. This novel instrument greatly expands the spectrum of biomarkers that can be measured by incorporating the capability of electron impact ionization with the previously demonstrated MALDI measurements. A prototype of this instrument about the size of a suitcase has been operating for several years. A new, smaller TOFMS with a 3" long reflectron or flight tube has been developed specifically to look at biological molecules.

- Detection and Analysis

The TOFMS has demonstrated the detection and analysis of urine biomarkers such as insulin-like growth factors (IGF-I), urinary 3-methylhistidine, estradiol, creatinine, and trivalent hydroxypyridinium cross-links. Measurement of such materials is important to both the Bone and Muscle Teams. The detection of compounds and biomarkers in both blood and saliva has also been demonstrated. Using a separate collection system, the breath from human subjects has also been analyzed.

During the period, the project team has designed and built a portable gas chromatograph-mass spectrometer (GC-MS) system. The GC-MS system will allow the monitoring of spacecraft for chemical and biological contaminants in addition to the analysis that can be performed on human fluids. The team completed studies on oxidative stress biomarkers for early detection of oxidative damage to DNA including detection at needed physiological monitoring levels. A study of Zolendronate (a bone loss countermeasure) is continuing. Melatonin studies are underway using direct measurement of the urinary melatonin metabolite by TOFMS.

Project 4: Improved Bubble Detection for EVA
PI: J. C. Buckey, Jr., M.D.

The objective of the Improved Bubble Detection for Extra-Vehicular Activity (EVA) project is to improve EVA efficiency and safety through the *in-vivo* validation of two unique ultrasonic bubble-sizing and detection instruments that exploit bubble resonance (not Doppler) to transcutaneously detect and size intravascular and extravascular bubbles (stationary or moving) *in vivo*.

NASA presently utilizes in-suit intravascular bubble detection based on Doppler ultrasound as an early warning for the development of decompression sickness (DCS). Doppler-based systems, however, can only detect moving, relatively large bubbles and provide little information about bubble size. The ability to size bubbles, detect stationary bubbles, and detect bubbles smaller than conventional Doppler-based systems may provide important information for early DSC detection and prevention. Creare Incorporated, under two large projects for NASA, has developed two ultrasonic bubble sizing and detection instruments intended for transcutaneous detection and sizing of: (1) intravascular bubbles in the size range of 30-200 μm , and (2)

extravascular bubble detection and sizing in the size range of 1 to 10 μm . The intravascular bubble-sizing instrument has been validated extensively *in vitro* using tissue phantoms that accurately mimic transcutaneous operation, and it has been successfully applied in a preliminary *in-vivo* trial. The extravascular bubble-sizing instrument is currently under development and has already demonstrated an ability to detect bubbles down to 1 μm in size *in vitro*. The instrument is presently being tested and optimized *in vitro* using a tissue phantom to simulate transcutaneous tissue bubble detection. Although these instruments have both been tested extensively *in vitro* and some preliminary but encouraging *in-vivo* work has been conducted with the intravascular bubble detection device, the potential for *in-vivo* applications of these instruments has not been fully explored nor have they been fully validated *in vivo*. The goal of the current project is to utilize these instruments to validate and optimize their performance *in vivo* and to begin to address several important long-posed DCS research questions.

One longstanding DSC concept is that gas phase nuclei exist normally in tissues before and after decompression. However, current Doppler-based monitoring techniques only allow for intravascular detection of relatively large bubbles, and as a result, little is known about the fundamental development of these bubbles. If, in fact, nitrogen bubbles normally exist in tissue and could be detected there, this instrument offers the potential to monitor DCS in a novel way, and potentially monitor the growth or disappearance of bubbles during decompression, recompression, or during oxygen pre-breathe. Such a capability could greatly enhance our ability to: (1) understand DCS, (2) detect the earliest stages of DCS, and (3) improve the efficacy and efficiency of strategies for mitigating the risk of DSC such as oxygen pre-breathing.

Accomplishments:

- Instrument Development

A dual-frequency ultrasound bubble detection instrument is under development. Bubbles insonified at two different frequencies act as nonlinear mixers and generate signals at both the sum and differences of the two frequencies. It has been shown that the signals (sum and difference) are enhanced if one frequency corresponds to the resonant frequency of the bubble. This phenomenon allows sizing of the bubbles. Two detection techniques are being explored: (1) single pulse (analyze FFT from a relatively long-duration signal) and (2) multiple pulse (analyze undersampled signal from multiple short-duration pulses). The team has shown that the single pulse method has greater sensitivity while the multiple pulse technique offers greater frequency and spatial resolution.

- Analysis (*in vitro* and *in vivo*)

In general, sum signals are detected when bubbles are present and are absent when bubbles are not present. Strong sum signals have been observed in excised tissue. Sum signals have been consistently detected at certain anatomical locations (e.g., the hip). Studies indicate no significant time dependence of sum signals occur with both adynamic and decompression thickness. Significant progress has been made in the creation of bubbles in gelatin as a tissue simulate. The team has demonstrated transthoracic bubble detection with bubble size discrimination using detected signals that are 6 to 10 times higher than the detector system's noise floor. Intravascular DCS bubbles in swine were studied using a frequency sweeping technique to interrogate bubbles of different sizes. Thus, histograms of the bubble sizes during

the progression of DCS have been produced. Studies with use of perfluorocarbon have also been conducted.

Project 5: Scanning Confocal Acoustic Diagnostic (SCAD) System for Bone Quality Assessment
PI: Y-X. Qin, Ph.D.

The goal of this project is to develop a new technology for monitoring bone quality of humans on Earth and during long-term space missions. This will lead to a better understanding of the progressive adaptation of bone loss in both aging populations and astronauts subject to microgravity. The principal objective of this project is to develop a portable scanning confocal acoustic diagnostic (SCAD) system capable of generating non-invasive, high-resolution ultrasound (US) attenuation and velocity maps of bone, and thus determining the relationship between ultrasound parameters and bone mineral density (BMD), bone quality, and other bone physical properties (i.e., stiffness and modulus). This system is relevant not only for ground-based determination of bone's physical properties, but can effectively be used in the space environment to determine subtle changes in density and strength during extended flights. In the proposed work, we will validate the structure and density information detected by SCAD using μ CT and mechanical testing methods in *ex vivo* animal models. Correlations to *in vivo* DEXA data derived from humans will also be made. The system can monitor the degree and risk of bone loss in space and on Earth, and serve as a major step towards clinic usage as an early diagnostic tool for osteoporosis.

Accomplishments:

During this period, the research team was focused on technology development of the SCAD system and on determining interrelationship between ultrasound determined parameters and micro BMD and architectural parameters in a quantitative manner. Bone quality is predicted via the correlation between SCAD determined data and μ CT identified BMD, porosity, trabecular space and trabecular width, as well as modulus using a large number of trabecular bone samples.

- Instrument Development

An experimental SCAD system has been successfully implemented, which is capable of generating non-invasive, high-resolution ultrasound attenuation and velocity maps of trabecular and cortical bones for predicting the risk of osteoporosis and fracture (U.S. patent pending). Spatial distributions of ultrasound parameters, i.e., ultrasonic attenuation and velocity, in the focal region in the trabecular bone can be measured and calculated, and thus it can be converted to an image, e.g., gray scale or virtual color. These new methods represent a major advance in investigating the mechanical properties of materials by utilizing confocal ultrasonic technology at appropriate frequencies. In addition, the ultrasound resolution and sensitivity are significantly improved over current ultrasound approaches.

A 3-D ultrasound scanning system consists of a pair of focused ultrasonic transducers (transmitting and receiving), digitally controlled moving stages, an ultrasonic wave generator unit, a preamplifier unit, and data acquisition unit with hardware. Ultrasonic scanning is program-controlled by a custom-written computer algorithm. The ultrasound propagates through tissue via water or acoustic gel. The converged beam can be as fine as 0.3 mm in diameter, through which most of the acoustic energy passes through the region of interest within the focal

region of the trabecular bone. Thus, the influences of soft tissue, cortical bone and irregular shape surfaces can be greatly reduced. The resolution of the beam can be adjusted up to 0.1 mm or less for each incremental step. In this confocal scanning mode, ultrasound parameters, i.e., BUA and UV, can generate a spatial acoustic map at the region of interest.

It is critical that acoustic map can be achieved in a fast scanning mode, i.e., testing bone quality during the space mission. With new design using dedicated CPU board and micro-controller, acoustic scan can be achieved in a continuous matter, which reduces the scanning time more than 10-fold compared to initial design. The scan time for a 2-D regional map is dependant on the scan resolution and the moving stage speed and digital response. For example, a 40 x 40 pixel 2-D array requires approximately 3 minutes of scan time, which originally required more than 30 minutes to complete the same scan.

The system, through the use of ultrasound confocal scanning mitigates the difficulties resulting from current ultrasound apparatus. In particular, focusing energy at a confocal point increases the sensitivity of a received signal, thereby reducing the noise due to the interaction of the soft tissue with the cortical shell and thus increasing the resolution. The device then makes it possible to make a highly reliable prediction of the material properties and density of a specimen through analytical and experimental calculation and correlation. The generated image of both ultrasound attenuation (UA) and velocity (UV) will typically comprise of discrete elements (pixels) each having specific values relating to image data at the individual part of the bone. From the image data, bone mineral density (BMD) and stiffness can be calculated. A set of computer algorithms was developed including the functions to calculate the interrelationship between acoustic parameters, the bone stiffness and density in a region of interest using linear and/or non-linear regression analyses.

- Bone Structure Determination

It is hypothesized that musculoskeletal disorders, e.g., osteoporosis, change not only the structure and the mineral density (BMD), but also the modulus and stiffness of bone. Advances in ultrasonic techniques provide an intriguing and a physical modality for characterizing not only the structural but also the material properties of bone in a manner of non-invasive, non-radiation, non-destructive, safe and relatively accurate. These changes in bone properties can be evaluated using the combination of micro-CT, mechanical testing and ultrasound mapping. While ultrasound provides only the acoustic wave propagation parameters when waves pass through the specimens, it becomes essential to propose a relationship between detected acoustic signals and bone properties. As an initial database, SCAD determined US velocity and attenuation are correlated with micro-CT identified BMD, porosity, trabecular thickness and trabecular space, as well as material bulk modulus determined through *ex vivo* animal bone tests.

A total of 61 sheep trabecular bone cube specimens (1 x 1 x 1 cm), were harvested from the distal femoral condyle. These were scanned in three orthogonal directions: longitudinal, med-lat and ant-post to determine UV and BUA. In comparison, bone's trabecular mineral density was determined by micro-CT measurements with a spatial resolution of approximately 30 micron (n = 17). Bulk mechanical modulus of the cubes was determined by a contact compressive mechanical testing in a MTS machine in all three orthogonal directions (n= 44). To predict bone's structural and strength properties, a series of regression analyses were conducted between the results of μ CT determined BMD, and stiffness, and the results obtained from the ultrasound measurements.

Through a micro-CT 3-D reconstruction, a number of parameters, such as the total volume (TV), bone volume (BV), bone mineral content (BMC), and BV/TV, were determined. These data show that the ratio of BV/TV in the normal group was $54.48 \pm 2.3\%$ and $49.45 \pm 3.5\%$ in the osteopenia group. These results have demonstrated a spatial difference among the samples, which is difficult to detect by normal ultrasound methods.

Latest tests involve scanning of healthy and osteoporotic patients on both the SCAD and a commercial dual energy x-ray absorptiometry (DEXA) system. Reasonable correction between ultrasound scanning at the calcaneus versus DEXA measurement at the hip.

Project 6: Heavy Ion Microbeam and Micron Resolution Detector
PI: V. Radeka

The use of high-energy microbeam provides a unique way to control the number of particles traversing individual cells and localizing the dose within the cell. High-energy heavy charged particles transfer their energy to biological organisms through high-density ionization and excitation along the particle track even with uniform irradiation. This characteristic of microscopically non-uniform dose delivery is expected to induce complex DNA damage and mutagenesis. This is contrasted to the relatively uniform dose delivery produced by gamma rays or electron beam irradiation. To investigate the distinct biological effects of heavy ions, especially to determine the effects of occupational and environmental exposure of very low doses of heavy charged particles (e.g., since virtually no cells receive more than one traversal cosmic ray HZE particle in its lifetime in a spaceflight environment), one approach is to select cells with the desired exposures from a randomly irradiated population.

Therefore, the goal of the Heavy Ion Microbeam/Detector project is to design and test a high-energy microbeam apparatus and a micro-resolution solid-state detector for space radiobiology studies. Such a facility permits heavy-ion radiobiology to address specifically the impact of signal transduction between cellular compartments as well as issues related to intercellular communication at limiting low fluences where not all the cells in a population have been traversed by even a single particle. Moreover, a high-energy ion microbeam will permit researchers to address an important unanswered question: whether neurons that survive traversal by HZE particles develop changes as a late consequence of the damage they incurred. Therefore, these low-fluence studies promise to aid in our understanding of the consequences of exposure to high-LET radiation such as encountered in the space radiation environment. The project involves the development of two major tools:

1. A microbeam of heavy ions (e.g., iron) at energies higher than at existing microbeam facilities (up to 3 GeV/nucleon). The microbeam would have a sufficiently small diameter (about 10 micrometers) to localize the ions to a single cell.
2. An electronic position sensitive detector for heavy ions with a position resolution better than 1 micrometer, to localize the position of ion impact within a particular region of the cell.

These developments will advance significantly the state-of-the-art of high-energy heavy ion microbeams and of high-resolution heavy ion detectors. For the cell studies employing these tools, the necessary infrastructure will include a micropositioning stage with a microscope and auxiliary detectors.

Accomplishments:

- Detectors

Various detector designs were evaluated including double-sided strip detectors, pixelated detectors, and the alternating stripixel detectors (ASD). The stripixel detector was selected as the most promising approach for making a high-resolution detector. The ASD consists of individual pixels alternately connected by X and Y readout lines (strips). The advantages of the stripixel detector are:

- (1) Both the pixels and strips can be made with a very small pitch in both directions (few micrometers). A few micrometer pitch (8-9 μm) allows submicron position resolution in two dimensions.
- (2) The narrow strips (2 μm) produce low leakage currents and capacitance.
- (3) The ASD also have flexibility to work with various readout schemes.

The simulation of the ASD chip design has been completed and the first prototype detector has been fabricated. While not completely functional, the first chip demonstrated that the desired patterning and resolution of features could be achieved. The large area test structures were functional. Some interconnection problems (opens) existed between first and second level metal. A new double-level metal process was developed that remedied the open contact problem.

A second batch of detectors has been recently fabricated with success. Detector chips with both 8 μm and 20 μm pitches are being assembled with preamplifiers and prepared for beam tests with heavy ions. Fabrication is complete on most of the detector electronics, including a new digital centroid finding module.

Project 7: Design of a Dynamic Exercise Countermeasures Device (DECD)
PI: B. L. Davis, Ph.D.

The objective of this study is to design and develop an exercise device that primarily counteracts microgravity-induced bone loss and muscle atrophy. Secondary benefits will include alleviating some of the problems associated with vestibular and cardiovascular adaptations to microgravity. This dynamic exercise device is based on data previously collected under NASA grant NAGW-5006.

The DECD was designed and constructed by Foster-Miller Incorporated. The assembly is designed as an exercise machine that allows the subject to simulate jumping in a microgravity environment without subjecting the surrounding vehicle structure to any significant impact loads. The assembly configuration is based on the conservation of the momentum principle whereby the subject "jumps" on a platform with mass similar to that of the subject. Both the subject and the platform are mounted on two coaxial support rails. They are both free to move relative to each other along these rails. The subject and the platform are connected by a pair of sleeved adjustable force springs. These springs are tensioned to produce a force that approximates the subject's body weight in a gravity environment. The subject is restrained on a torso support carriage. He places his feet on the push-off plate and pushes the counterbalance assembly away by straightening his legs. This stretches the force springs and simulates standing in a gravity environment. The subject then bends his knees and "jumps" off the platform. This jumping force causes the guided subject and the guided counterbalance assembly to separate.

The two masses, traveling in opposite directions, will be decelerated by the force applied by the interconnecting adjustable force springs. The deceleration rates will be proportional to the masses. When motion has stopped, the two masses will accelerate towards each other. The subject will "land" on the platform and soften the landing force by bending his knees. Both moving masses will decelerate to zero. This is equivalent to jumping in a gravity environment. This jumping cycle and the primary forces generated and reacted within the exercise machine assembly are balanced and isolated from the exterior environment. This arrangement will not be sensitive to mass differences between the loaded carriages. When subjected to the same force, both will have the same momentum with compensating differences in accelerations and velocities.

The primary strength of this project is that the DECD targets multiple systems of the body that are adversely affected by prolonged microgravity. The exercise countermeasure device that we are developing will provide physical stimuli to bones, muscles, the cardiovascular system and, most likely, the vestibular system. Another strength of the project lies in its simplicity. Astronauts will perform jumping exercises while they are tethered to a support platform. The principle of conservation of momentum dictates that when an astronaut pushes off the platform, he/she will experience a "flight" phase followed by an impact phase when "landing" occurs and there is contact with the support platform. The device is being designed with key considerations for mass, and for this reason the support platform will consist of an empty chamber that will be filled once the spacecraft has reached its orbit, and minimizing unbalanced forces and vibrations that are transmitted to the spacecraft.

Using the DECD: (1) astronauts will be able to exercise without the need for uncomfortable harnesses (since we generate the same forces under the feet with tether forces of 50% bodyweight as we do with 100% bodyweight tensions), (2) the hardware will be considerably lighter than the treadmill flown on previous missions (60 kg compared with 320 kg), and (3) future research will need to focus on the in-flight benefits of jumping exercises versus high frequency vibrations that are applied through the undersurface of the feet.

Accomplishments:

Significant progress on the DECD has been made. The design and fabrication of the second (or working) prototype is complete. The dynamic exercise countermeasure device (DECD) configuration has been sized for a user population with body weights between 54 and 89 kg and standing heights between 162 and 175 cm. The device measures 305 cm in length, 89 cm wide, and 47 cm tall. Empty weight is 60 kg. The general arrangement includes a pair of side rails positioned to be coplanar with the combined user/carriage center of gravity and the loaded platform center of gravity. The platform tank is being sized to achieve a platform-loaded mass of 82 kg. The DECD includes a load adjustment feature in the form of a hand crank and indicator which will allow the user to pre adjust the standing load force experienced between user and platform between 40% and 60% of the user body weight. A total of four shock cords each with a free length of approximately 460 cm long are being used to develop the standing force. Shock testing is currently underway to select the optimum length of shock cord required to satisfy the specified load range. To date, forces exceeding the average walking peak load (on Earth) have been achieved by the DECD in at least two exercise cycles (maximum jumping and jump rope).

Project 8: Space Qualifiable MRI System
PI: I. N. Bankman, Ph.D.

The objective of the Space Qualifiable MRI System project is to develop a proof-of-concept engineering model of a Magnetic Resonance Imaging (MRI) system for small animal models and possible astronaut limbs that can be space qualified. Small animal MRI systems, although commercially available, are too massive to be considered for spaceflight, with masses >1000 kg and power requirements of >5 kW. Availability of a flight qualified MRI could especially benefit the study of physiological alterations in the space environment and the development, verification, and maintenance of countermeasures. The countermeasure development efforts of the following NSBRI teams would be significantly enhanced: Bone Loss, Cardiovascular Alterations; Muscle Alterations and atrophy; Neurovestibular Adaptation; Nutrition, Physical Fitness and Rehabilitation; Radiation Effects; DNA Damage and Repair, and Smart Medical Systems. This will establish a new era in space physiology research complementing and evaluating the effectiveness of the hind limb suspended mouse and rat models and human bed rest studies that are routinely carried out on Earth. Frequent MRI scanning of mice and rats in space is especially important to many of the research teams for understanding the basic processes at work.

The major goal of this project is to develop an engineering model of an MRI system for human limbs and small animals, specifically mice and rats, to demonstrate that a flight qualified system can be fabricated with the following characteristics:

- Field strength >1 Tesla and perhaps as high as 1.5 Tesla.
- Field inhomogeneity ≤ 8 ppm over spherical imaging volume of 10 cm diameter and ≤ 10 ppm out to 15 cm.
- Imaging of small animals (mice and rats).
- Standard resolution mode giving a resolution of 234 microns over a spherical imaging diameter of 6 cm for mice and rats.
- Higher resolution mode giving a resolution of 117 microns over a spherical imaging diameter of 6 cm for mice and.
- Mass <150 kg.
- Average power in standby mode <1 kW and during normal use <1.2 kW.

The proposed design is a compromise between field strength, imaging volume, system mass, system average power, and the ability to image small animals with sufficient resolution.

Accomplishments:

The MRI Project began in July of 2002, after a change of PI and some refocusing of project goals. The project conducted a detailed trade-off study between image resolution and both the magnet bore size and strength subject to a weight constraint of less than 150 kg. A design for the prototype MRI has been completed and the unit constructed using a commercial magnet that does not meet the size and weight requirements of the final MRI. The use of the commercial magnet has allowed the system to be checked out and facilitated the development of image quality metrics.

General Team Accomplishments:

The NSBRI Technology Development Team is characterized as an integrated, multidisciplinary group chartered to develop systems, instrumentation, devices, and algorithms. The accomplishments noted above provide a clear demonstration that this objective has been achieved. In addition to this, the project teams have demonstrated unique capabilities of being able to structure and accomplish complex applied research and development. An Annual Technology Development Team Retreat was held on September 15, 2003. Some of the characteristics and accomplishments that cross project boundaries are:

- The capability to successfully conduct rapid system prototyping.

All of the Technology Development Team projects were successful in accomplishing the goal of developing and demonstrating prototype system implementations. A number of patent disclosures and/or applications have resulted from the developments. The ability to support the development of practical and useful tools in support of basic research requirements is a necessary element of a successful undertaking such as the NSBRI.

- The capability to transition developments to practical embodiments.

As an extension of the prior item, it is not sufficient to develop unique, one-of-a-kind prototypes. The developments must have practical means of supporting the basic research efforts by providing reliable and robust tools. The Technology Development Team projects have successfully demonstrated the ability to transition their developments to the real-world environment. For example, the AMPDXA scanner is ready to support human testing and commercialization efforts are underway. The SCAD system is ready for human testing and a prototype dynamic exercise device has been built.

- The capability to network and collaborate with NASA, the medical community, etc.

All of the Technology Development Team projects have established close and on-going interactions with NASA and the medical community. The interactions were initiated during the project proposal phase to assure that the intended development addressed a current space issue and was founded in a practical medical basis. The interactions have experienced positive growth and expansion throughout the research and development cycle. The result of the networking is that the resultant development products have validated utility to the space and medical communities. And, the networking within the communities has provided very good exposure and visibility for other applications and opportunities. A Technology Working Group meeting was held in January 2002 at the NSBRI retreat. At this meeting, over 35 opportunities for technology and instrument development were identified. Another Technology Working Group meeting is planned for January 21004 at the NSBRI retreat.

- The capability to produce quantifiable results to support countermeasures research.

The basic research programs of the NSBRI are charged with developing and evaluating countermeasures to the effects of long endurance exposure to microgravity. This effort requires that cause and effect relationships be identified and characterized. Proper characterization mandates that empirical data be referenced to a standard and be quantitative in nature. All of the Technology Development Team projects have achieved a level of standardization and

quantitation that is necessary to support the basic research initiatives. In fact, some of the engineering models that have resulted from the team's activities exceed the accuracy and precision found in existing clinical and commercial systems (e.g., the AMPDXA and the SCAD).

Implications of Accomplishments

The seven active NSBRI Technology Development Team projects are making significant progress against their development goals. The three continuing projects have operating instruments, while the relatively new projects are in various phases of instrumentation development. For example, the two AMPDXA instruments (LTB and HTB) are operational and the HTB is has begun human trials. In addition, commercialization discussions are underway to transfer the technology to industry. The neutron spectrometer is being readied for a high altitude balloon flight after completing several successful aircraft flight tests. The TOFMS has identified with high sensitivity several biomarkers associated with bone and muscle loss. The heavy ion microbeam and detector system has been designed and the detector fabricated. When completed, this resource will address several very current and significant issues in cellular biology. The SCAD system is operational and is performing correlation studies between measurements in the extremities and the weight-bearing bones. The bubble detection project has developed prototype equipment that has detected microbubble formation at various nucleation sites within the body. The DECD is in its second prototype design phase. Each of the projects and prototype instruments is addressing the major goals of the Technology Development Team, the NSBRI, and NASA by either developing research tools that facilitate the measurement and analysis of critical parameters necessary for the research of the other NSBRI teams or creating direct countermeasures to the risks encountered in long-duration spaceflight.

The Technology Development Team is constantly on the alert for technological developments and advancements (Goal 1) that will have impact on both the NSBRI and space life science research. For example, working closely with the Bone Team, the Technology Development Team was able to identify two major impediments to the development of countermeasures for bone demineralization: understanding of the bone mineral loss process and being able to monitor the instantaneous conditions of the subjects' bones. The TOFMS (Project No. 3) has been adapted to monitor the biomarkers for bone loss. While the TOFMS was developed under DARPA funding for solids analysis, newly invented methods of sample preparation and fixing techniques has allowed its applicability to biological specimen analysis. Historically, space-based monitoring of such biomarkers has typically relied on collection of specimens (urine, blood, etc.) and then storage of the specimens until Earthly return. Specimen analysis may, under good conditions, be completed many months after completion of the mission, but certainly does not afford the ability to provide closed-loop monitoring and control of countermeasures (Goal 2).

The AMPDXA project (No. 1) and the SCAD project (No. 5) specifically address the monitoring issue. Both these projects have completed engineering model instrumentation developments (Goal 2) and have demonstrated the ability to provide quantitative information that is critical to the current and future research of the Bone Team. These devices have been designed to be directly adaptable for in-space use. Size, weight, and power are currently, or soon will be, appropriate for routine launch and regular use on-orbit or in missions beyond Earth. Using advanced automation techniques (Goal 1), these devices and their associated analysis methods can be operated by individuals with very little training. Thus, the devices have broad utility in both space- and Earth-based applications.

The bone demineralization conditions that astronauts experience in space are similar to those that exist in clinical populations (e.g., age-related osteoporosis, quadriplegic, etc.) on Earth. Thus, the research supported by the AMPDXA, SCAD, and TOFMS is expected to have a direct positive influence on health care delivery on Earth (Goal 3). In addition, the technology itself has demonstrated better performance than commercially available devices. In particular, commercialization (Goal 4) of a clinical version of the AMPDXA is being pursued that has great potential to improve screening and treatment for age-related osteoporosis.

Muscle alteration research faces the same challenges of loss mechanism determination and monitoring as noted above for bone. Both the AMPDXA and TOFMS provide the same capabilities (i.e., monitoring and biomarker determination, respectively) in support of risk reduction for muscle as they do for bone. Advanced AMPDXA muscle algorithms (Goal 1), coupled with a radical new x-ray source (Goal 1), offer a promise of similar precision measurements of muscle as has been demonstrated for bone. The DECD (Project No. 7) offers the potential to directly countermeasure muscle loss in space (Goal 2).

Exposure to radiation in space is a threat that can lead to an increased risk of cancer and DNA damage. A significant portion of the exposure, between 30-60%, results from neutron sources that are extremely difficult to monitor, let alone characterize, in real-time. The absence of a portable, quantitative, real-time neutron spectrometer results in an exposure safety risk for astronauts (Goal 1). The Neutron Energy Spectrometer Project (No. 2) is developing a spectrometer (Goal 2) that can supply information on the neutron environment to the Radiation Effects Team in support of assessing radiation damage and cancer risk. The prototype of this unit is operational and has completed several flight tests on F15 or F18 aircraft as well as long-duration, high-altitude balloon flights. The last flight in October 2003 was extremely successful. The Ion Microbeam Project (No. 6) will address radiation damage at the cellular level.

Orthostatic intolerance can result in syncope when an individual is subjected to gravitational influence after exposure to microgravity. This situation can pose severe risks to astronauts who have to execute unassisted emergency procedures or extraterrestrial landings. The need to predict, prevent, or control orthostatic intolerance and its effects is significant to the space program. Both the TOFMS and the MRI project (No. 8) have the ability to provide near real-time monitoring of parameters related to orthostatic intolerance. The TOFMS can detect various heart-related biomarkers and the MRI will be able to monitor soft tissue, including vein and artery blood volumes and fluid shifts, in animals and potentially on the extremities of the astronauts.

To identify and appropriately fund these pipeline projects requires constant interaction between the NSBRI research teams and the Technology Development Team. To promote this integration, which satisfies much of Goal 5, the Technology Development Team established the Technology Working Group (TWG) as a formal mechanism for this liaison. Meetings of the TWG have been conducted frequently with expanded participation from academia, industry, and government. In addition, Technology Development Team participation in the annual retreats of the other teams also fosters cooperation and the synergism necessary to identify the technical requirements (Goal 1). Such input from NSBRI research teams, coupled with strong industrial and academic input, allows the Technology Development Team to develop calls for research that address technological solutions for the risk factors associated with long-duration spaceflight that are in concert with the established research goals of the other NSBRI teams. Part of the strategic growth plan for the Technology Development Team is the ability to direct key technology

development efforts in addition to or in conjunction with the projects received in response to the research announcements. Such responses may leave gaps in the envisioned technology development requirements. Past meetings of the TWG have identified over 35 instruments or technology development needs of the space research community that are not currently being addressed by the NSBRI Technology Development Program. These gaps offer opportunities to foster important research that could accelerate the overall space effort. Support sources for such selected top-down driven research will have to be developed.

Appendix E



SUMMARY OF INSTITUTE ACTIVITIES

**Board of Directors Meeting
March 27, 2003**

The Institute has made steady progress in its countermeasures development research and educational programs over the past six months. Several new initiatives have been launched, and there has been an internal management reorganization to better prepare the Institute for new opportunities and challenges in meeting its mission.

The community has been affected by the tragic loss of the crew and science of STS-107. The brave men and women of the Shuttle Columbia are deeply missed.

Advances in NSBRI science and technology, to ensure crew health and minimize the hazardous biomedical risks of space travel, include the maturation of several imaging and sensing devices for monitoring physiological and medical status. The Institute is providing NASA with unique capabilities to enhance the Clinical Status Evaluation (CSE), which is becoming the gold standard in assessing the health of *all* crew on international space missions.

Through a joint NSBRI/NASA Steering Committee that meets bi-weekly, the Institute is developing and implementing ways to integrate its research teams with NASA scientists and medical operations personnel. The aim is to reduce prioritized biomedical risks for different mission scenarios through a process that links hypothesis-driven research to medical operations using the CSE.

Team leaders have been active in establishing an increased presence at Johnson Space Center and guiding their teams in emphasizing research at higher countermeasure readiness levels. Productivity in this regard was evident at the Bioastronautics Investigators Workshop in January 2003 and in the list of accomplishments in the FY02 Annual Report delivered to NASA in November 2002.

Four new ground-based projects have been added to the research portfolio in the areas of nutrition and exercise, human performance, smart medicine and muscle alterations. Funding for one of the projects was awarded by the French agency CNES.

The External Advisory Council (EAC) membership has been updated and the Board of Scientific Counselors has been revitalized. The Institute has worked closely with NASA in the development of a research announcement scheduled for release on April 15, 2003. This announcement is important since the tenure of the current Team and Associate Team Leaders expires in FY04, and investigators must competitively apply to have proposals and leadership applications awarded.

NASA has launched a new initiative in radiation, and there is a need to readjust the Institute's approach toward radiation research. While this change has come about quickly, there is an opportunity to strengthen our ties to other NASA radiation investigators and to better tap NASA's investment in Brookhaven National Laboratory, as one of our consortium institutions. An NSBRI/NASA Radiation Steering Group has been established, and some of the issues facing us in linking radiation to the other teams were discussed at the recent EAC meeting.

The February 2003 EAC meeting provided an opportunity to have the Team Strategic Plans vetted before Council and to have research priorities re-examined in the context of an upcoming research announcement. Future directions for the Education and Public Outreach Team were also reviewed, and the Institute plans to launch its fellowship program in FY04.

The Institute has completed a revised draft of its Strategic Plan and has forwarded its *Policy on Team Leadership* to NASA, in response to an action item recommended in the June 2002 Review of the NSBRI Strategic Research Plan. NASA and NSBRI have also been revising the original 1997 Cooperative Agreement Management Plan to better align it with the evolution of the two parties and their partnership. The 25% cap on total ground-based NASA biomedical research funding that is available to the NSBRI is being lifted.

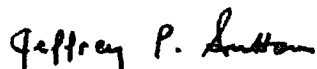
Notice of budget stability at \$30M per annum of core support for FY03-FY07 was received from NASA in a Cooperative Agreement letter in October 2002. Amounts of support above this core award are possible through several means, which the Institute is already implementing.

Budget stability is essential as the Institute moves forward with its program planning and management expansion to better address needs. Information technology and bioinformatics efforts have, and will continue to be, bolstered. An increased emphasis on metrics and productivity will be occurring. Importantly, new management positions in science integration and in medical operations at the NSBRI/NASA interface will be added to integrate and capitalize on the unique partnership.

With the resignation of the Associate Director, a Search Committee has been formed and an active search for a new Associate Director is under way. In the interim, the Institute's new Chief of Staff has assumed several of the administrative duties requiring immediate attention.

The next six months promises to be an exciting time in the Institute's history. There will be a closer working relationship between NASA and NSBRI at all levels of the Institute. There is good support for ground-based biomedical research at NASA, and the Institute performs a critical function for NASA in countermeasures research and development. With science re-planning for the International Space Station and with increased emphasis on the CSE, the Institute is well positioned to make advances that will mitigate health risks during long-duration human space flight.

Respectfully submitted,



Jeffrey P. Sutton, M.D., Ph.D.
Director



SUMMARY OF INSTITUTE ACTIVITIES

Board of Directors Meeting September 18, 2003

The Institute continues to have solid scientific productivity and success in its research and education programs. The teams are performing well and progress has been made in coordinating NSBRI research and development projects with NASA's programs in countermeasure evaluation and validation, and in space medicine. This effort, which is overseen by the NSBRI/NASA Steering Committee, has begun to forge a pipeline that will allow promising investigations within our Institute to advance to space analog testing and flight.

The Institute's Revised Strategic Plan has been well vetted and signed off by NSBRI and NASA. It is posted to our public Web site, along with the Team Strategic Plans and a growing resource of materials that reflect the depth and breadth of Institute activities. There are approximately 80,000 hits per month to the site.

We used a secure electronic process for managing NSBRI proposals submitted in response to NRA 03-OBPR-04, an open grant proposal solicitation for ground-based countermeasure research. There was an excellent response, and we anticipate significant program continuity, given the proportion of current projects that are undergoing competitive renewal and the fact that many of the current team leaders have applied to continue in leadership roles. Substantial effort has been put into budget planning to ensure that momentum, flexibility and productivity are sustained for countermeasure development.

Metrics have been developed and implemented for our core research program, as well as for projects performed at Johnson Space Center (JSC) by members of our NSBRI/NASA translational workforce. This workforce is supported by non-core funds managed by NSBRI, and as part of our integration efforts, JSC-based translational research is being incorporated into our teams, where there is a clear advantage to do so.

The task of reconstituting the Board of Scientific Councilors (BSC) is well under way. There is now at least one BSC member per discipline area, with the goal of having two members per area. BSC members are appointed for five years to serve on joint NASA/NSBRI peer review panels and provide one source of corporate memory for the Institute. Importantly, the BSC is available to peer review synergy proposals that arise between research announcement cycles and can accommodate review of targeted solicitations, such as calls for program project grants to answer specific critical questions on the Bioastronautics Critical Path Roadmap (BCPR). The BSC will also evaluate streamlined progress reports, using new metrics aligned with the BCPR and including measures of intellectual property developed in coordination with the Industry Forum.

As research and development projects advance, it is apparent that gaps in transitioning countermeasures to operations and space medicine exist. The Institute is taking a proactive stance in filling those gaps. For example, researchers on the Human Performance Factors, Sleep and Chronobiology Team discovered that it is not only the duration, timing and intensity of light, but also the wavelength, that is important in entrainment of circadian rhythm. It turns out that blue spectrum light is superior to other wavelengths in sustaining performance and is therefore a potential countermeasure in the space environment. This finding has significant Earth-based benefits as well. Through NSBRI efforts with human factors experts at JSC, who are part of the translational workforce, a link has been established to operations and potential implementation in crew quarters on the ground and in space.

Similarly, the Neurobehavioral and Psychosocial Factors Team is tasked to deliver products to the JSC Behavioral Medicine group to help reduce the high-priority BCPR risk of poor psychosocial adaptation in space. However, there is no formal bridge at the NSBRI/NASA interface in this important area. Therefore, the NSBRI has recruited an experienced research psychologist from the Department of Defense to establish a bidirectional link between NSBRI research teams and NASA operations and evidenced-based medicine (where appropriate).

To help ensure the Institute develops operationally valid countermeasures, a flight surgeon has been assigned to each research team. We also have begun to engage members of the User Panel. Furthermore, we are pleased that Jonathan Clark, M.D., former chief NASA flight surgeon, has filled the position of Space Medicine Liaison. Dr. Clark will be working with the Director and team leaders to accelerate countermeasure development and help focus relevant projects, given the changing opportunities for analog testing and flight.

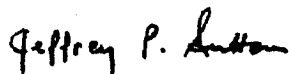
NSBRI participated in ultrasound imaging of the current International Space Station crew and in imaging training for the next crew. A portion of a peer-reviewed ultrasound flight experiment has been added to our research portfolio with funding derived from non-core sources. NSBRI language has been included by NASA in recent non-NSBRI research solicitations, thereby increasing our pivotal role in Bioastronautics.

Our Teacher Academy, which trains teachers from every state in space life sciences, has been widely acknowledged as a success. These teachers, in turn, train other teachers with a cumulative outreach to more than 100,000 students. The Institute had an excellent group of summer interns this year, who received research experiences at JSC and Johns Hopkins University. Two new Houston/JSC seminar series – one in space biomedical research and one in clinical space medicine – have been launched and are run jointly with the JSC Space Medicine and Health Care Systems Office.

The Associate Director search is proceeding on schedule and two candidates are under consideration following the Committee interview stage. In the interim, our Chief of Staff, Mrs. Kathryn Bruning, has done an outstanding job with the NSBRI Headquarters managers and staff, as well as with members of our multiple constituencies.

There are several projects in development, including the establishment of the NSBRI Advanced Projects Laboratory near JSC, which would allow investigators from the NSBRI academic community to be present onsite to advance their countermeasure projects and interact with operational personnel. Involvement of team leaders and the External Advisory Council in strategic planning and tactical implementation, to capitalize on opportunities, remains strong. The postdoctoral training program is in the final preparation stages and will commence in FY 04. The Institute is well positioned for continued success, and by remaining focused on our mission and providing answers to important critical questions, will have high impact for reducing the biomedical hazards of human space travel.

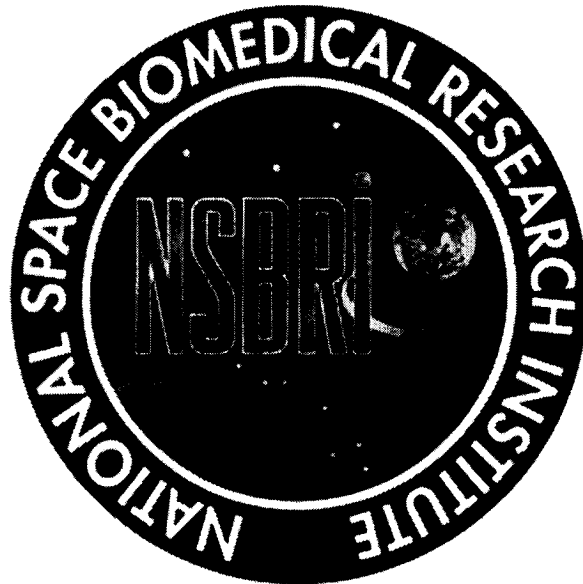
Respectfully submitted,



Jeffrey P. Sutton, M.D., Ph.D.
Director

Appendix F

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



**Core Research Program
Publications and Presentations List**

October 1, 2002 – September 30, 2003

National Space Biomedical Research Institute Publications

Bone Loss Research Team

Articles

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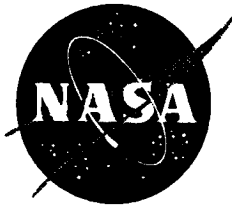
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Appendix G



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National Aeronautics and Space Administration
Office of Biological and Physical Research
Washington, DC 20546-0001

NASA Research Announcement

Soliciting Ground-based

Research Proposals

Biomedical Research and Countermeasures Program

- 1. Independent Investigator Research Projects, or**
- 2. Research Projects for a Research Team of the National Space Biomedical Research Institute**

A Research Announcement for the
NASA Office of Biological and Physical Research

Notices of Intent Due: May 15, 2003
Proposals Due: July 15, 2003

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**NASA Research Announcement:
Ground-based Research Proposals**

Biomedical Research and Countermeasures Program

- 1. Independent Investigator Research Projects or**
- 2. Research Projects for a Research Team of the National
Space Biomedical Research Institute**

Summary and Supplemental Information

NASA's new Vision for the 21st century is:

*To improve life here,
To extend life to there,
To find life beyond.*

The Office of Biological and Physical Research's (OBPR) contribution to the Agency is to realize this Vision, written as a Mission Statement, that motivates our research on the ISS and is the framework for the activities of OBPR:

Humans will extend the exploration of space. To prepare for and hasten the journey, OBPR must answer these questions through its research:

- How can we assure the survival of humans traveling far from Earth?
- What must we know about how space changes life forms, so that humankind will flourish?
- What new opportunities can our research bring to expand our understanding of the laws of nature and enrich lives on Earth?
- What technology must we create to enable the next explorers to go beyond where we have been?
- How can we educate and inspire the next generations to take the journey?

OBPR is developing a Research Plan that will provide a top-level description of the direction that the Enterprise will take to answer these questions and fulfill its mission. The OBPR Research Plan can be accessed at: <http://spaceresearch.nasa.gov/>

This National Aeronautics and Space Administration (NASA) Research Announcement (NRA) solicits ground-based proposals for the Biomedical Research and Countermeasures (BR&C) Program. The BR&C Program sponsors research that will lead to the development of countermeasures against the negative effects of space flight on humans. Sponsored research may

be performed at “Countermeasure Readiness Levels” (CLRs) from basic research (CRLs 1-3) through evaluation and validation (CRLs 7 & 8). Applicants should refer to Figure 1 in Appendix A for a detailed description of the CRLs. Each applicant must identify what CRL their application addresses. This NRA solicits research proposals for either **independent investigator research projects** (see Appendix B for details), or proposals for the opportunity to become a **member of an integrated countermeasure development team** of the National Space Biomedical Research Institute (NSBRI) (see Appendix C for details). Please note that proposals for the NSBRI Radiation Effects team and independent investigator research projects in radiation **are not** being solicited in this NRA. Proposals that synergistically bridge multiple disciplines for the purpose of modeling the effects of microgravity on the human body to aid in the development and testing of countermeasures, or to develop technologies that enable research in one or more NSBRI research areas are strongly encouraged.

Applicants must determine if their proposed research is best suited to be conducted independently or as part of an integrated research team of the NSBRI. **Do not submit the same research proposal to both opportunities.**

The Biomedical Research and Countermeasures Program is interested in supporting independent investigator research projects in CRL range 1-7 with a majority of the funded tasks residing in the lower CRL range. The NSBRI is primarily interested in supporting research, in the CRL range of 3-7, that will participate as part of a countermeasure development team focused on advancing the research towards an applied intervention that can be evaluated and validated by the Countermeasure Evaluation and Validation Project (CRL 7 & 8).

NASA investigators use the space environment to increase knowledge of biological and medical processes, to provide the biomedical foundation in support of the International Space Station and exploration beyond low Earth orbit, and to enrich life on Earth through the transfer of new space technology, medicine, and fundamental knowledge. This research supports NASA’s mission through the Office of Biological and Physical Research (OBPR). All respondents to this NRA are strongly encouraged to promote general scientific literacy and public understanding of life sciences, the space environment, and the OBPR programs through formal and informal education opportunities. Where appropriate, supported investigators will be required to produce, in collaboration with NASA, a plan for communicating their work to the public.

In this NRA,

- Appendix A provides an introduction and overview to the goals, objectives, and implementation strategies of the BR&C Program.
- Appendices B and C contain descriptions of the two opportunities, and specific instructions for submitting a notice of intent (NOI) and instructions for proposal submission.
- Appendix D contains copies of the certifications required to be followed with any signed application.
- Appendix E contains the standard Instructions for Responding to NASA Research Announcements.
- Appendix F provides sample copies of the forms that must be used when preparing the application. Please note that Forms A-E are only required for Independent Investigator

Research Projects applications. Form F is required for Independent Investigator Research Projects and NSBRI Research Team applications.

The BR&C Program and the NSBRI share scientific and educational goals to fund research that will result in the delivery of health-related countermeasures for astronauts. NASA is committed to maintaining a strong, openly competitive, peer-reviewed research program. Proposals submitted in response to this NRA must address the research emphases described in this Announcement. Those that do not will be returned. **This NRA does not request proposals for flight research. Proposals that require flight resources will be returned to the proposer without being reviewed.** Other NRAs calling for focused research or utilization of unique resources may be issued throughout the year.

Proposals for individual investigator grants selected by NASA will be funded incrementally as grants for activities lasting up to four years, pending satisfactory progress. Proposals selected by the NSBRI will be funded as subawards by the NSBRI for activities lasting up to four years. The funding duration will depend on proposal requirements, review panel recommendations, and continuing progress of the activity. All proposals will be evaluated for overall scientific and technical merit by independent peer review panels. Relevance to NASA's programmatic needs and goals will be evaluated by NASA. Relevance to the NSBRI's programmatic needs and goals will be evaluated separately by the NSBRI. Final selection will be coordinated between the Bioastronautics Research Division at NASA Headquarters and the NSBRI to ensure programmatic balance and to eliminate duplicate efforts. Funds are not currently available for awards under this NRA. The government's obligation to make award(s) is contingent upon the availability of appropriated funds from which payment can be made and the receipt of proposals that the government determines are acceptable for award under this NRA. The total annual cost for ground research should not exceed \$450,000. NASA and the NSBRI do not provide separate funding for direct and indirect costs; thus, the amount of the award requested is the total of all costs submitted in the proposed budget. It is planned for selections to be announced by December 15, 2003, and awarded shortly thereafter.

Inclusion of Women and Minorities in Research Involving Human Subjects – NASA and the NSBRI have adopted the NIH policy regarding this matter. Women and members of minority groups and their subpopulations must be included in NASA-supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research.

Participation in this NRA is open to all categories of organizations, industry, educational institutions, other nonprofit organizations, NASA laboratories, and other agencies of the U.S. government. NASA will not fund non-U.S. institutions. The NSBRI accepts and reviews proposals from foreign applicants, but potential foreign applicants should note that, normally, the country of origin, not the NSBRI, will fund applications from non-U.S. organizations. Potential foreign applicants should coordinate their application with both the NSBRI and the appropriate funding agency in their own country.

A Notice Of Intent (NOI) to propose is requested by May 15, 2003. Proposals must be submitted by July 15, 2003, 5:00 p.m. Eastern Time (see Appendices B and C of this NRA for specific instructions for these activities).

The following items apply only to this NRA:

Solicitation NRA Identifier:	NRA 03-OBPR-04
Number of Copies Required:	Original + 20 paper copies for the non-NSBRI submissions; electronic proposals for the NSBRI submissions
Notices of Intent Due:	May 15, 2003
Proposals Due:	July 15, 2003
Selection Announcement:	December 15, 2003
Funding Begins:	Approximately 30-90 days following notification of selection
Selecting Officials:	For individual BR&C proposals: Director, Bioastronautics Research Division, Office of Biological and Physical Research, NASA Headquarters For NSBRI proposals: Director, National Space Biomedical Research Institute

Additional information about the BR&C Program and independent investigator proposals is available from

David L. Tomko, Ph.D.
NASA Headquarters, Code UB
Washington, DC 20546-0001
Telephone: 202-358-2211
Fax: 202-358-4168
Email: dtomko@nasa.gov

Information about the NSBRI and its existing research teams is available from

Jeffrey P. Sutton, M.D., Ph.D.
Director, National Space Biomedical Research Institute
One Baylor Plaza, NA-425
Houston, TX 77030-3498
Telephone: 713-798-7412
Fax: 713-798-7413
Email: director@www.nsbri.org

All prospective proposers to this NRA are advised that the highest priority in all of NASA's programs is given to safety and mission assurance, occupational health, environmental protection, information technology, export control, and security. NASA's safety priorities are to

protect (i) the public, (ii) astronauts and pilots, (iii) the NASA workforce (including employees working under NASA instruments), and (iv) high-value equipment and property. All proposals submitted in response to this solicitation are expected to comply with this policy.

Grants Office points of contact will be identified in selection letters. This NRA will be updated and issued annually and is NASA's primary means of obtaining ground-based biomedical research proposals from the life sciences community. This NRA is restricted to the programs named above and described in detail in the Appendices. Potential investigators should read with care the program descriptions that are of interest, and focus their proposals on the specific research emphases defined in this NRA.

Your interest and cooperation in participating in this effort is appreciated.

Original signed by

Mary E. Kicza
Associate Administrator
Office of Biological and Physical Research

Background Information

Opportunities for Ground-based Research in the Biomedical Research and Countermeasures Program

I. Introduction

This NASA Research Announcement (NRA) is a consolidated NASA solicitation for research proposals in support of the goals and objectives of the NASA Office of Biological and Physical Research (OBPR). Ground-based research is solicited for conduct by the Biomedical Research & Countermeasures (BR&C) Program. Proposers may apply for a grant as an independent investigator, or as a member of one of the research teams of the National Space Biomedical Research Institute (NSBRI).

The goals of this program are to

- develop an understanding of the physiological mechanisms responsible for space-flight-related biomedical and behavioral changes in humans in support of countermeasure development;
- develop countermeasures that allow humans to live and work in microgravity for long durations, minimize the risks in readapting to gravity, and optimize crew safety, well-being, and performance; and
- identify, characterize, and mitigate (prevent and reduce) health, environmental, and other operational human medical risks associated with space exploration.

The BR&C Program is responsible for sponsoring research that will lead to development of practical health-related methods for the prevention, diagnosis, treatment, and/or rehabilitation of humans who live and work in microgravity. It also responds directly to the requirements, approved by the Office of the Chief Health and Medical Officer, which deal with the health and safety of human space travel (see *Guidance for NASA Medical Board Procedures*, Bibliographic reference #8 of Appendix A).

The NSBRI is a NASA-initiated and -funded private, non-profit research consortium charged by NASA with developing biomedical countermeasures for potential health problems that could occur in astronauts either during long-duration space flight or on their return to Earth. The NSBRI's current program consists of approximately 90 research and technology projects organized into 11 research teams.

It is critical for investigators to read carefully all of the instructions in this NRA. All proposals will undergo peer review using similar processes and procedures, but procedures and forms for proposal submission differ for the different programs and elements, and the

eventual funding of selected proposals will differ for the different types of awards. Programmatic balance is maintained by the selecting official(s) for the program.

The research programs described in this NRA support the utilization of specialized NASA ground-based facilities and the development of special technologies required in the pursuit of its research goals. Investigators can access NASA specialized ground-based facilities for their research. Please refer to the *Space Life Sciences Ground Facilities Information Package* for instructions on how to incorporate the use of these facilities into a proposal is online at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/index.html.

This NRA does not request proposals for flight research. Proposals that require flight resources will be returned to the proposer without being reviewed. It is important that the proposer read all instructions in this NRA carefully, as many of the programmatic emphases are different from those appearing in previous NRAs. In addition, each Appendix includes guidelines, requirements, and instructions for preparing and submitting proposals, and defines the administrative policies governing the particular components described in this NRA.

II. Critical Path Roadmap (CPR)

In order to identify and make publicly known the biomedical risks of space flight, and the research questions that must be answered to reduce those risks, NASA has developed the Critical Path Roadmap (CPR). The CPR is an interdisciplinary tool to assess, understand, mitigate, and manage the risks to humans that are associated with long-term exposure to the space environment. It assumes an overarching strategy that integrates requirements, risks, risk factors, critical questions, tasks, deliverables, and risk mitigation with the intent of directing biomedical research in support of human space flight, especially human missions of exploration. The CPR is based in part on recommendations from internal NASA experts, NSBRI scientists, advisory committees representing the United States science community, task forces, and published reports such as the National Research Council (NRC) Space Studies Board's "A Strategy for Research in Space Biology and Medicine in the New Century;" the Aerospace Medical Advisory Committee; the NASA Task Force on Countermeasures; the International Space Life Sciences Working Groups publications on Radiation, Bone, Muscle, Cardiovascular, Human Factors, and Neuroscience Workshops; and the NASA Medical Policy Board Document.

The ultimate goal of the CPR is to protect the health and safety of space flight crews by allowing NASA and the community of scientists to better define and focus the research that is required for development and validation of operational health care "deliverables" for the prevention, treatment, and rehabilitation of space flight changes and of appropriate habitation and medical care systems.

The current CPR identifies 55 risks and 250 critical questions. A more extensive overview, as well as a list of all the risks and critical questions for the CPR, should be reviewed by potential investigators at <http://criticalpath.jsc.nasa.gov/>

The proposer must examine and understand the CPR, and specify in their proposal the rationale and evidence underlying which risks and critical questions their proposed research will answer. An example is shown in Table I below, and the blank form (Form F) can be found in Appendix F. A similar assessment will be performed by NASA and the NSBRI to understand how the proposed research addresses the CPR risks and critical questions. Proposals that do not identify what CPR risks and questions are being addressed by the research will be returned to the proposer without review.

The Biomedical Research and Countermeasures (BR&C) Program utilizes annual and final reports to assess progress relative to stated research objectives and hypotheses as declared in the original grant proposal by the Principal Investigator. It is critical that the reports indicate how the investigation relates to critical questions outlined in the CPR. It must be understood by proposal authors that reporting of progress on an annual basis shall be required and shall be linked to CPR risks and critical questions. In addition, the final report shall address the entire scope of the project rather than the final year and shall be linked to CPR risks and critical questions.

TABLE I

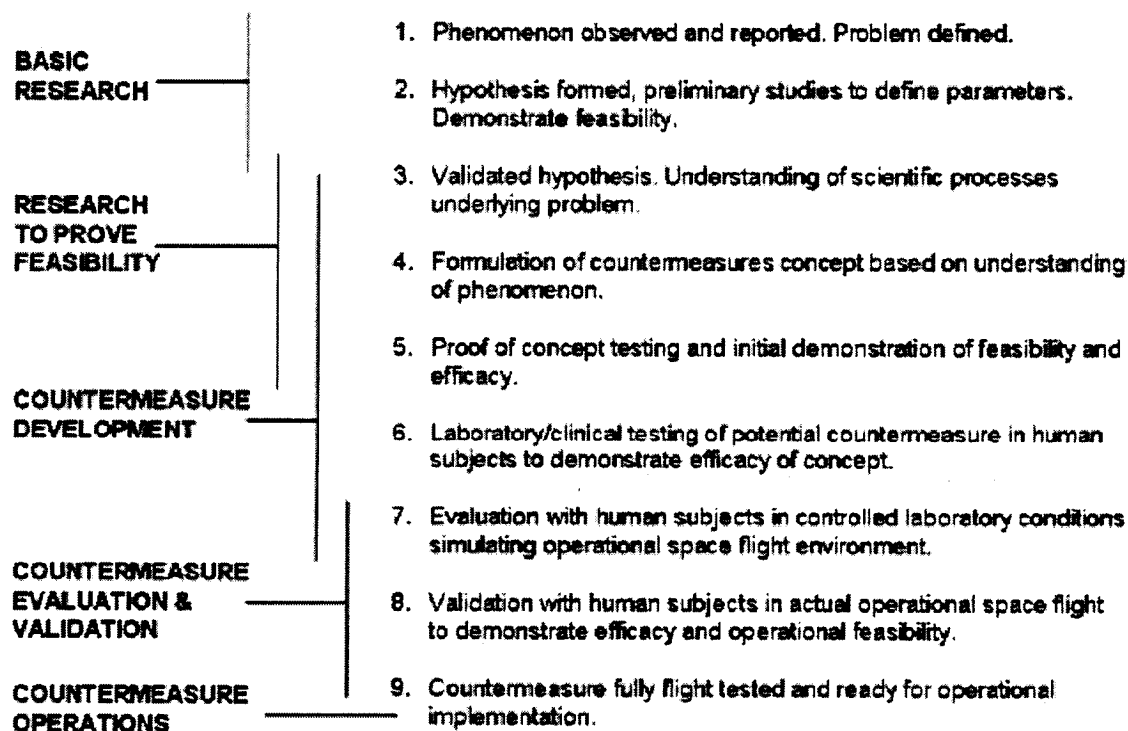
EXAMPLE ONLY – Complete Form F in Appendix F for specific proposal

Hypotheses	Risk Number (from Critical Path Roadmap)	Critical Question Number (from Critical Path Roadmap)	Critical Question (from Critical Path Roadmap)	Specific Aim
The combined effects of hypergravity (simulating launch and landing) and bedrest (simulating space flight) along with associated physical and psychological stress will decrease virus specific cellular immunity and reactivate latent herpes viruses.	Risk #22 Immunodeficiency, Infections	7.03	Do factors associated with flight (stress, environment, micro-gravity, nutritional status, radiation) affect humoral or cell mediated immune function, non-specific immunity, mucosal immunity, or immune surveillance capabilities of crewmembers in a manner that exposes them to unacceptable medical risk (disease, allergy, delayed wound healing)?	#1: Assess stress levels utilizing measures of biochemical and psychological stress. #2: Determine virus specific T-lymphocyte immunocompetence.
	Risk #22 Immunodeficiency, Infections	7.04	Do factors associated with spaceflight increase disease pathogens by activation of latent viruses?	3: Quantify latent herpes virus reactivation
Additional hypotheses as required.				

III. Countermeasure Readiness Levels (CRL)

NASA's Biomedical Research and Countermeasures (BR&C) Program has developed a scale to allow NASA and the NSBRI to define, assess, and quantify the level of "countermeasure readiness." The use of this scale allows Program Managers to determine and describe how each funded research project fits into the countermeasure development "flow" and to monitor progress in countermeasure development. This section describes this scale and how it is used. **Each investigator must examine and understand the CRL scale and specify in the proposal the CRL that will result from the funding and conduct of their proposed research.** Figure 1 illustrates the CRL scale, which describes the level of scientific maturity of BR&C research from the fundamental studies that suggest potential countermeasures to studies that allow the systematic evaluation and validation of countermeasures ready for operational implementation.

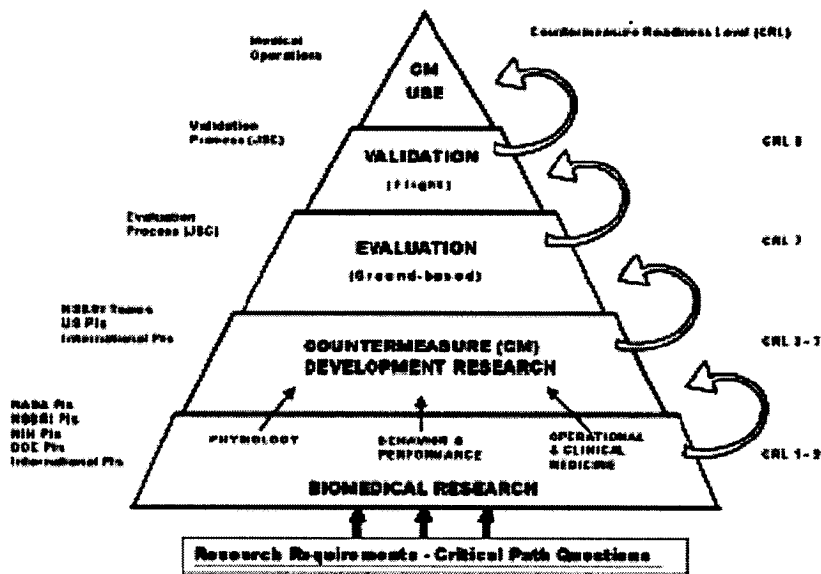
Figure 1. Countermeasure Readiness Levels



Countermeasure development usually progresses through systematic research. Research flows through various levels of countermeasure readiness. Figure 2 represents this general progression. The boundaries between the types of activities are approximate. A potential countermeasure ready for validation in flight is one that has a thorough, successful history of ground-based, clinical, and/or flight analog testing. This NRA does not solicit CRL 7 and 8 research sponsored

by the Countermeasure Evaluation and Validation Project. Other NASA Research Announcements may be issued throughout the year to call for studies to evaluate potential countermeasures. To have NASA notify you by email in the future about its requests for research proposals, register at <http://proposals.hq.nasa.gov/proposal.cfm>

Figure 2. Countermeasure Development Process



IV. Biomedical Data

Biomedical data are being collected in both the Longitudinal Study of Astronaut Health (LSAH) and the Life Sciences Data Archive (LSDA). These databases can be made available for research activities subject to scientific merit review, ethical issues related to the protection of subjects, and privacy issues. Identifiable human medical and research data is only available with the consent of the astronaut and/or research subject. Additional information can be obtained from Victor S. Schneider, M.D. (email: vschneider@nasa.gov or phone: 202-358-2204)

The LSAH is an electronic database of medical information collected over the active career and post career life of the astronauts. Data are also available on a comparison group matched to the astronauts at a 3:1 ratio by age, sex, and initial body mass index. The data recorded include annual and flight related medical evaluation and medical debriefs following space flights for astronauts and routine annual medical evaluations for the comparison group.

V. Review and Selection Process

This Appendix supersedes, modifies, or extends the requirements enumerated in Appendix E. All proposals must comply with the general requirements of the Announcement as described in both Appendices A and E. Appendices B and C contain specific requirements and explanations

for each opportunity above and beyond the NASA-specified requirements. Appendix E outlines the NASA-specified requirements for proposal submission and should be used for clarification and reference. Upon receipt, proposals will be reviewed for compliance with the requirements of this Announcement. This includes the following:

1. Submission of complete proposals specified in this Announcement. Proposals must be responsive to the areas of program element emphasis described in this Announcement and include a project description that is not more than 20 pages in length.
2. Submission, as specified in Appendices B and C to investigators, of appropriate Institutional Review Board (IRB) or Animal Care and Use Committee (ACUC) certification for all proposals using human or animal test subjects.
3. Submission of a budget within the guidelines specified in this Announcement and for a funding period not exceeding four years in duration.
- 4) Proposals that are revised versions of proposals previously submitted to NASA or the NSBRI **must be clearly designated** as such on the proposal cover page, and **must contain an explanation** of how the revised proposal has addressed criticisms from the previous NASA or NSBRI review. This explanation must be presented in a separate section of no more than two pages at the beginning of the project description, and is in addition to the 20 pages allowed for the project description. Related changes to the research plan should be highlighted in the body of the project description.
- 5) Submission of all other appropriate forms as required by this NASA Research Announcement (refer to Appendix F).

Note: Non-compliant proposals will be withdrawn from the review process and returned to the investigator without further review.

Compliant proposals submitted in response to this Announcement will undergo an intrinsic scientific or technical merit review. Only those proposals most highly rated in the merit review process will undergo the additional reviews for program relevance and cost. It should be noted that in order to achieve program balance, specific topics that are currently well represented in the BR&C Program portfolio will be de-emphasized. Investigators are encouraged to review summaries of the research currently funded in this program by accessing the Office of Biological and Physical Research Program Tasks and Bibliography (OBPR Task Book) at:

<http://research.hq.nasa.gov/taskbook.cfm>

Scientific or Technical Merit Review

A merit review of proposals submitted to this NRA will be conducted by panels of scientific or technical experts. A single set of discipline-specific panels, administered by NASA Peer Review Services, will evaluate all proposals submitted to this NRA. The number and diversity of experts required will be determined by the response to this NRA, and by the variety of disciplines represented in the proposals relevant to the research emphases described in Appendix B and C of this NRA. Merit review panels will *score proposals from 0-100*.

The scientific merit score assigned by each panel *will not be affected by the proposed cost of the work, nor will it reflect the programmatic relevance of the proposed work to NASA*. However, the panels will be encouraged to include comments concerning the proposal's budget and relevance to NASA or the NSBRI in the critique of each proposal, after it has been scored.

All of the following will be used in determining the merit score:

- **Significance:** Does this study address an important problem? If the aims of the application are achieved, how will scientific knowledge or technology be advanced? What will be the effect of these studies on the concepts, methods, or products that drive this field? What is the likelihood that the proposed research will lead to new countermeasures or tests of the utility of countermeasures? Is there a significant societal or economic impact?
- **Approach:** Are the conceptual framework, design, methods, and analyses adequately developed, well-integrated, and appropriate to the aims of the project? Is the proposed approach likely to yield the desired results? Does the applicant acknowledge potential problem areas and consider alternative tactics? Are there strong interdisciplinary components?
- **Innovation:** Does the project employ novel concepts, approaches, or methods? Are the aims original and innovative? Does the project challenge existing paradigms or develop new methodologies or technologies?
- **Investigator:** Are the scientists in the project, including collaborators, suitably trained for the proposed work? Is the work proposed appropriate to the experience level of the principal investigator and other researchers (if any)? Is the evidence of the investigator's productivity satisfactory?
- **Environment:** Does the scientific environment in which the work will be performed contribute to the probability of success? Do the proposed experiments take advantage of unique features of the scientific environment or employ useful collaborative arrangements? Is there evidence of institutional support?

Evaluation of Programmatic Relevance and Cost

A **second review** of proposals will evaluate the programmatic relevance and cost of all proposed work. Evaluation of the cost of a proposed effort includes consideration of the realism and reasonableness of the proposed cost and the relationship of the proposed cost to available funds. Is the proposal responsive to the needs of NASA or the NSBRI *as expressed in this NRA*? Programmatic relevance will include an evaluation of how the proposed work may help achieve an appropriate balance of scientific and technical tasks required by critical research issues faced. Evaluation of programmatic relevance will vary according to the specific element of this NRA. NSBRI proposals will undergo a subsequent evaluation and scoring as to how well the proposal matches the NSBRI team's strategic plan. NSBRI proposals will be reviewed and prioritized by members of the NSBRI's Board of Scientific Counselors (BSC) on how well the proposal matches the NSBRI team's strategic plan. The results of the merit review and the BSC strategic

plan assessment will be forwarded to the NSBRI's External Advisory Council (EAC) for selection recommendations to the NSBRI Director. See Appendix C, Section V for more information on the NSBRI selection process.

Development of a Selection Recommendation

A selection recommendation will be developed based on the merit review, programmatic relevance, and cost, as described above. The most important element in the evaluation process is the merit review, which carries the highest weight in final evaluation and selection. The other factors are approximately equal in weight to each other. **Deficiencies in any one of these factors may prevent selection of a proposal.** The development of selection recommendations is the responsibility of NASA for the individual proposals submitted to the Biomedical Research and Countermeasures (BR&C) Program. The development of selection recommendations is the responsibility of the NSBRI for proposals submitted to the NSBRI elements of this NRA. Selections for funding of individual BR&C proposals will be made by the Director of the Bioastronautics Research Division, Office of Biological and Physical Research (OBPR), and selection of NSBRI proposals will be made by the NSBRI Director. Final selection will be coordinated between the Bioastronautics Research Division at NASA Headquarters and the NSBRI to ensure programmatic balance and elimination of duplicate efforts.

NASA and the NSBRI reserve the right to select and make an award covering only a portion of an investigator's project. The applicant will be given the opportunity to accept or decline such a partial award. If two or more proposals address similar problems and/or adopt similar approaches, NASA or the NSBRI may request that the investigators consolidate specific parts of their projects into a single project and work as a team. The selection review may also recommend changes in the way in which a specific proposal should be funded (i.e., as an individual investigator award or as a member of an NSBRI team). Acceptance of such a recommendation shall be at the discretion of the Principal Investigator. If a proposal submitted to NASA is found to be more appropriate to satisfy the NSBRI requirements, the Principal Investigator will be expected to become a full member of the appropriate NSBRI team. Any negotiations with a Principal Investigator will occur only after the peer review of proposals has been completed. Only grants will be awarded as a result of this NRA.

VI. Program Reporting

It is expected that results from funded research will be published in peer-reviewed journals as the work is completed. Published papers must acknowledge NASA or NSBRI support. In addition, investigators whose proposals are selected must also provide annual reports on progress in achieving the goals of the research project.

Final Report. A final report is required that shall include a summary of completed research and a record of all scientific communications and peer-reviewed publications to date. This report must be submitted to the NASA Technical Monitor or to the NSBRI within 90 days after the end of the grant period.

Annual and final reports shall emphasize the relevance of research results to the CPR risks and questions defined in Table 1 in the original proposal. It should be noted that the final report shall incorporate the results and relevance to Table 1 for the entire duration of the research project.

VII. Support of Education and Outreach

OBPR envisions that the selected individual investigator proposals will be structured and operated in a manner that supports the nation's educational initiatives and goals (including support of historically black colleges and universities and other minority universities), and in particular the need to promote scientific and technical education at all levels. OBPR envisions that the selected proposals will support the goals for public awareness and outreach to the general public (see Appendix E). The selected investigators are invited to participate in OBPR-funded educational programs.

Because the NSBRI has a unique team approach to experimental science, it has a separate education and public outreach component that adheres to these OBPR policies. For information on NSBRI's Education and Public Outreach Program, go to <http://www.nsbri.org/Education/>

OBPR Policy for Education (6-12) and Public Outreach

The proposal represents an opportunity for NASA to enhance and broaden the public's understanding and appreciation of the value of OBPR research in the context of NASA's mission. Therefore, all principal investigators are strongly encouraged to promote general scientific literacy and public understanding of OBPR research through formal and/or informal education opportunities. If appropriate, proposals should include a clear and concise description of the education and outreach activities proposed. Examples include such items as involvement of students in the research activities, technology transfer plans, public information programs that will inform the general public of the benefits being gained from the research, and/or plans for incorporation of scientific results obtained into educational curricula consistent with educational standards. Where appropriate, the supported institution will be required to produce, in collaboration with NASA, a plan for communicating to the public the value and importance of their work.

Once NRA selections are made, the selected PI's will have an opportunity to request additional funding through an OBPR-sponsored pilot program to implement an education outreach program at the grades 6-12 level, at an amount not to exceed \$10,000 per year for the term of the grant. A request for proposal will accompany the selection notification letter. Proposals will be due within 60 days of selection notification and shall be limited to 4 pages. A review of these proposals by educational specialists will determine which proposals will be funded.

For more information, the OBPR Educational Outreach Vision, Mission, Goals and Operating Guidelines are provided in the Educational Outreach handbook. The handbook is available on the Internet at: http://spaceresearch.nasa.gov/research_projects/nrahardware.html. If you would like assistance in preparing outreach proposals, the National Space Grant College and Fellowship

Program is available to help. Visit their website at <http://education.nasa.gov/spacegrant> to identify the state-by-state listing of Space Grant Directors.

VIII. Bibliography

1. **OBPR Program Tasks and Bibliography (Task Books)** for FY1995-2002 are available at <http://research.hq.nasa.gov/taskbook.cfm>
2. **National Space Biomedical Research Institute Web site.** <http://www.nsbri.org/>
3. **Space Life Sciences Ground Facilities Information Package.** This document is available at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/index.html
4. **Guidebook for Proposers Responding to a NASA Research Announcement (NRA), 2003 edition:** <http://www.hq.nasa.gov/office/procurement/nraguidebook/>
5. **Life Sciences research publications:** <http://spaceline.usuhs.mil/>
Additional information may be obtained from the SPACELINE Project (phone: 301-295-2482; email: spaceline@usuhs.mil).
6. **The Space Life Sciences Data Archive (LSDA)** is an online database containing descriptions and results of completed NASA-sponsored flight experiments. Descriptions are included of experiments, missions, procedures, hardware, biospecimens collected, personnel, and documents. Biospecimens that are available for research purposes are described in detail. A limited number of experiments contain final reports and spreadsheet data suitable for downloading. Data from human subjects are unavailable online for reasons of privacy.
Internet address: <http://lsda.jsc.nasa.gov/>
LSDA Help Desk: 281-483-7876
Email: lsda@semail.jsc.nasa.gov
7. **Center for Advanced Studies in the Space Life Sciences** contains a list of workshops and seminars sponsored by the Center. The proceedings and final reports of these workshops are also posted as they become available at <http://www.mbl.edu/CASSLS/workshops.html>
8. **Guidance for NASA Medical Board Procedures.** National Aeronautics and Space Administration, Medical Policy Board. Richard Williams, M.D., Chairperson. NASA Headquarters. This document is available at http://peer1.nasaprs.com/peer_review/prog/mpbhand.pdf
9. **A Strategy for Research in Space Biology and Medicine in the New Century.** National Academy of Science. National Research Council Committee on Space Biology and Medicine. Mary J. Osborn, Committee Chairperson. 1998. Washington DC: National Academy Press. <http://www.nas.edu/ssb/csbn1.html/>

10. **Space Physiology and Medicine, 3rd ed.** A. Nicogossian, C. Huntoon, and S. Pool. (Eds.). 1994. Philadelphia, PA: Lea & Febiger.
11. **Task Force on Countermeasures.** This report incorporates the output of the Countermeasures Task Force, the Vestibular Countermeasures Task Group, and the Behavior and Performance Working Group into a unified document. This document is available at http://peer1.nasaprs.com/peer_review/prog/countermeasures/countermeasures.html
12. **International Workshop on Cardiovascular Research in Space.** *Medicine and Science in Sports and Exercise*, Volume 28, Number 10 Supplement, 1996.
13. **Muscle Research in Space: International Workshop.** *International Journal of Sports Medicine*, Volume 18, Supplement 4, S257-S331, 1997.
14. **Space Neuroscience Research.** *Brain Research Reviews*, Volume 28, Numbers 1/2, Special Issue, 1998.
15. **International Workshop on Bone Research in Space.** *Bone, Official Journal of the International Bone and Mineral Society*, Volume 22, Number 5 (Supplement), 1999.
16. **Small Clinical Trials: Issues and Challenges.** Institute of Medicine, National Academy Press, Washington, DC. <http://www.nap.edu/books/0309073332/html/>
17. **Sex and Gender: Exploring the Biological Contributions to Human Health.** *NIH Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, 59 Fed. Reg. 14508 (1994).*
18. **Grant and Cooperative Agreement Handbook.** Office of Procurement, National Aeronautics and Space Administration, Washington, D.C. 20546
19. **Safe Passage, Astronaut Care for Exploration.** Institute of Medicine, National Academy Press, 2101 Constitution Avenue NW, Washington DC 20418 (2001).
20. **NSBRI Team Strategic Plans:**
 Bone Loss: <http://www.nsbri.org/Research/Bone.html>
 Cardiovascular Alterations: <http://www.nsbri.org/Research/Cardio.html>
 Human Performance Factors: <http://www.nsbri.org/Research/Sleep.html>
 Immunology, Infection and Hematology: <http://www.nsbri.org/Research/Immune.html>
 Muscle Alterations and Atrophy: <http://www.nsbri.org/Research/Muscle.html>
 Neurobehavioral and Psychosocial Factors: <http://www.nsbri.org/Research/Psycho.html>
 Neurovestibular Adaptation: <http://www.nsbri.org/Research/Neuro.html>
 Nutrition, Fitness and Rehabilitation: <http://www.nsbri.org/Research/Nutrition.html>

Smart Medical Systems: http://www.nsbri.org/Research/Med_Sys.html

Technology Development: <http://www.nsbri.org/Research/Tech.html>

21. Space and Life Sciences Directorate Web site: <http://www.jsc.nasa.gov/sa/>

**NASA Research Announcement
Ground-based Research Proposals**

Biomedical Research and Countermeasures Program

Independent Investigator Research Projects

NOTE 1: This Appendix should only be used for scientists interested in conducting an independent investigator research project. Scientists interested in team-based research should see Appendix C.

NOTE 2: Proposals for radiation research are not being solicited in this NRA. A separate NRA soliciting proposals for radiation research will be released later this year. To have NASA notify you by electronic mail in the future about its requests for research proposals, register at <http://proposals.hq.nasa.gov/proposal.cfm>

I. Introduction

The emphasis of this solicitation for ground-based research studies performed by individual investigators is to develop insights into physiologic changes that are likely to occur as a consequence of extended periods of flight. The BR&C Program supports basic, applied, and clinical research by individual investigators. Researchers may use hypogravity simulation models (e.g., bed rest, unilateral lower limb suspension, tail suspension, etc.) or hypergravity produced by centrifugation for their research studies. Experiments may use human subjects, animal models, or other appropriate models in the development of countermeasures.

II. Emphases for Independent Investigator Research Projects

Research Emphases for FY 2003

This solicitation includes three elements, each focused on research that will lead to the development and ultimate use of countermeasures to the deleterious effects of space flight: 1) Physiology, 2) Behavior and Performance, and 3) Clinical Research in Support of Space Missions.

Mechanistic research is solicited that supports the development of ground-based biomedical countermeasures to the effects of space flight. A countermeasure to help astronauts is any means or procedural strategy that prevents or reduces the negative effects of space, or aids in the recovery upon return to Earth. It should be noted that the astronaut corps is diverse, comprised of men and women 30-60 years of age and of various ethnic backgrounds. Countermeasures should be robust enough to be efficacious across this population and be tailored for individual

specificity. **Integrated approaches that study interactions between different physiological systems in the design and application of potential countermeasures are encouraged.** Identifying the effects of experimental interventions on non-target systems as well as the targeted system is deemed to be of particular importance. Research to support the solution to operational and clinical problems is also sought.

It is expected that the average total annual (direct + indirect) cost of selected proposals will be \$250,000. The total annual cost of a single proposal should not exceed \$450,000.

1. Physiology

Proposals are requested for ground-based studies that will lead to a better understanding of the effects of space flight and exposure to microgravity on physiological function. Space physiology includes 1) fluid volume and cardiopulmonary, including cardiovascular alterations; 2) musculoskeletal, including bone loss and muscle alterations and atrophy; 3) neuroscience, including vestibular and sensorimotor function, and endocrine control; 4) immunology, infection, and hematology; 5) food, nutrition, and metabolism; and 6) integrative physiology; as well as 7) advanced technology development within the above elements. Studies that use integrated approaches are particularly encouraged. Proposals must represent questions and priorities enumerated in the Critical Path Roadmap at <http://criticalpath.jsc.nasa.gov/>.

Contact: Bruce M. Hather, Ph.D./Biastronautics Research Division
Telephone: 202-358-1796
Email: bhather@hq.nasa.gov

2. Behavior and Performance

The Behavior and Performance element of the program addresses issues of 1) perception and cognition; 2) human physical performance; 3) personal, interpersonal, and group dynamics (coping, response to stress, etc.); 4) habitability; and 5) sleep and circadian rhythms. Studies should be directed towards understanding the effects of space flight on behavior and performance.

This element includes experiments designed to understand the mechanisms by which microgravity, confinement, cumulative sleep loss, mission design and events, spacecraft environment, noise and light, and sensory/cognitive or sensorimotor changes affect the behavior and performance of flight crews and ground-support crews. It also addresses psychosocial, gender, and cross-cultural aspects of human missions in space. Studies of relationships between individuals and individuals in groups are also addressed. Proposers may utilize existing databases and ground simulations in extreme and isolated analogs and test beds used to extrapolate to responses that might be expected in long-duration space flight. Behavior and performance research priorities for ground-based studies include the following:

a. Psychological Research

Research is solicited that will lead to the development and validation of predictive tools for the assessment of psychological well-being, cognitive processing, mood, and emotion, especially as those are affected by multi-cultural and gender variables in long-duration space missions. Also of interest are hypothesis driven ground-based studies that would suggest and evaluate potential proactive techniques or strategies for reducing stress and improving well-being, mood, emotion, and cognitive processing in long-duration crews. Such techniques or strategies might include crew manipulation of environmental factors.

b. Psychiatric Issues

Research is required to understand how to detect and treat behavioral disorders that might occur in locations remote from usual health care facilities, e.g., during long-duration space flight.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. For a broad, detailed listing of NASA Life Sciences Behavior and Performance research priorities, the Countermeasures Task Force Report on Behavior and Performance can be obtained online at http://peer1.nasaprs.com/peer_review/prog/countermeasures/countermeasures.html

Contact: Bette Siegel, Ph.D./Bioastronautics Research Division
Telephone: 202-358-2245
Email: bette.seigel@nasa.gov

3. Clinical Research in Support of Space Missions (Medicine in Extreme Environments)

The Clinical Research in Support of Space Missions element of the program focuses on research that will lead to development of medical knowledge and technologies required to maintain human health and performance in space and on return to Earth. Medical knowledge must be expanded so that the practice of Space Medicine in the microgravity environment can be evidence-based. Medical and surgical procedures, treatment, and imaging systems are required to diagnose and treat illnesses and injuries that may occur in space. The Clinical Research in Support of Space Missions element of the program solicits research required to improve or answer specific questions about in-flight diagnosis, therapy, and postflight rehabilitation.

a. Diagnosis

Ground-based research analogs for space flight research are required to complete the understanding of the patho-physiology, diagnosis, and therapeutic modalities required for implementation of an evidence-based practice of Space Medicine. Proposals for the development of non-invasive diagnostic tests and autonomous and semi-autonomous patient monitoring systems are requested. Research is also sought for the development of medical information systems that support the onboard medical provider.

b. Therapy

High priority will be given to research proposals to study the mechanisms of changes that could occur during space flight in the therapeutic effectiveness and adverse drug interactions of medications for common illnesses. Proposals are sought for research to enhance surgical capabilities in space. High priority will be given to proposals that investigate the application of fiber optic-based and minimally invasive surgical techniques.

Proposals are sought in medical education focused on the development and maintenance of medical capabilities for both physicians and non-physician crew medical officers. Priority will be given to those research proposals that develop and test new training paradigms. Priority will be given to proposals that address the development of space flight treatment capabilities for acute medical and surgical emergencies such as wounds, lacerations, and burns; toxic exposures; decompression illness; and dental, ophthalmologic, urologic, gastrointestinal, and gynecologic emergencies.

c. Risk Evaluation

High priority will be given to research proposals that will evaluate whether the presence of a patent foramen ovale increases the medical risk (incidence and morbidity) related to decompression sickness.

d. Rehabilitation

Proposals are sought for research to develop more effective rehabilitation techniques for deconditioned space travelers on their return to Earth.

e. Pharmaceuticals and Blood Replacement Solutions

Proposals are sought for ground-based research that emphasizes efficacy and route of administration of pharmaceuticals, intravenous fluids, and blood replacement substances that are stored for extended periods of time and would be required for clinical care of patients in extreme environments (e.g., radiation resistant, storage at ambient temperature, small volume, etc.).

Proposals must represent questions and priorities enumerated in the Critical Path Roadmap at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of their proposal.

Contact: Victor S. Schneider, M.D./Bioastronautics Research Division
Telephone: 202-358-2204
Email: vschneider@nasa.gov

III. Application Procedures for Independent Investigator Research Projects

Except where specifically stated otherwise in this NRA, applicants must prepare proposals in accordance with the “Instructions for Responding to NASA Research Announcements, “ which is part of the NASA Federal Acquisition Regulations (FAR) Supplement (NFS), Part 1852.235-72 (Appendix E).

Instructions for Notice of Intent and Proposal Submission

A. SYS-EYFUS Registration for All Applicants

SYS-EYFUS is an electronic system used by NASA Headquarters to manage research solicitation activity, plan for the receipt of research proposals, track the receipt and peer evaluation of these proposals, and manage funded research (grants, cooperative agreements, etc.) sponsored by NASA’s Office of Equal Opportunity (Code E), Office of Earth Science (Code Y), Office of Human Resources & Education Division (Code F), Office of Biological and Physical Research (Code U), Office of Space Science (Code S), and the Office of Space Flight (Code M). SYS-EYFUS also supports the funding and administration of awards pursuant to selection of these research opportunities.

The SYS-EYFUS Help Desk is available at 202-479-9376. Help desk hours are from 8 a.m. to 6 p.m. Eastern time.

All investigators planning to submit a proposal to this solicitation are requested to register online with SYS-EYFUS. Comprehensive help, instructions, and contact information are provided online. SYS-EYFUS can be accessed at <http://proposals.hq.nasa.gov/>.

If you have previously registered with SYS-EYFUS, you are requested to verify and update your user information. If you have forgotten your user ID or password, select the “Forgot Your Password” option and type in your first and last name to search our database. The system will send an automatic email message with your username and password to the email address listed in our database.

B. Instructions for Preparing a Notice of Intent

All investigators planning to submit a proposal in response to this solicitation are requested to submit a **non-binding** notice of intent (NOI) to propose by the due date identified in the Summary and Supplemental Information Section of this NRA via the Web at the following address:

<http://proposals.hq.nasa.gov/proposal.cfm>

- 1) Login to SYS-EYFUS at the URL listed above and select “New Notice of Intent.”

- 2) The Division Specific Opportunities screen will appear. In the selection window, highlight **Bioastronautics Research Division** and click on “Continue.”
- 3) The List of Existing Opportunities screen will appear. In the selection window, highlight **03-OBPR-04** and then click on “Continue.”
- 4) This will bring you to the Notice of Intent Submission Form. **All fields are required.**
 - a. Please select from **only** the following three options: For the proposal type field on this form, new / no prior support means that the investigator has not received NASA funding from 1999 through 2002, new/prior support means that the investigator has received NASA funding between 1999 and 2002, and revised means that the proposal is a revised version of a proposal submitted to NASA and reviewed from 1999 through 2002, but not funded. A proposal previously submitted but not funded, should be identified as being “revised” even if the original Principal Investigator has changed.
- 5) Click on “Submit NOI Page.”
- 6) The Team Member Page screen will appear, where you can add or remove team members. Select continue if there are no other team members. To add a team member, highlight the role option on the selection list, type in first and last name and click on search. When the resulting set appears, choose the appropriate radio button and click on ADD to add the person to the NOI. After you are done, click on “Continue.” **IMPORTANT:** If the team member is not listed in our database, please have them add themselves as a new user to the system. You may then add them to your team member list.
- 7) After continuing from the Team Members Page, your NOI will be displayed. Click on “Resubmit NOI Page” to complete your NOI submission.
- 8) You may edit and resubmit your NOI at any time before the submission deadline of May 15, 2003. Once you submit an NOI, it cannot be deleted, only edited. For title, team member, or any other changes, please edit your existing NOI and resubmit changes to avoid duplicate records.

C. Instructions for Preparing and Electronically Submitting a Proposal Cover Page

All investigators planning to submit a proposal in response to this solicitation must electronically submit proposal cover page information online and provide a hardcopy of the cover page attached to each proposal copy by the due date indicated in the Summary and Supplemental Information Section of this NRA. The proposal cover page can be submitted and printed at

<http://proposals.hq.nasa.gov/proposal.cfm>

- 1) Login to SYS-EYFUS at the URL listed above.
- 2) To submit a New Proposal Cover Page, click the “New Proposal Cover Page” option on the SYS-EYFUS Options screen, and the New Proposals Cover Page screen will appear.
- 3) If you previously submitted an NOI in response to this solicitation, choose to carry over the existing NOI. This option will populate the cover page fields with the NOI information. Edit the information as necessary, click “Continue,” and proceed to #8 below.
- 4) If you did not previously submit an NOI, click on New Proposal Cover Page option, and the Division Specific Opportunities screen will appear.
- 5) In the selection window, highlight **Bioastronautics Research Division** and click on “Continue.”
- 6) The List of Existing Opportunities screen will appear. In the selection window, highlight **03-OBPR-04** and then click on “Continue.”
- 7) This will bring you to the Proposal Cover Page Submission Form. Fill in all the fields. All fields are required.
 - a. Please select from **only** the following three options: For the proposal type field on this form, new / no prior support means that the investigator has not received NASA funding from 1999 through 2002, new / prior support means that the investigator has received NASA funding between 1999 through 2002, and revised means that the proposal is a revised version of a proposal submitted to NASA and reviewed from 1999 through 2002, but not funded. A proposal previously submitted but not funded should be identified as being “revised” even if the original Principal Investigator has changed.
 - b. Indicate the status of IRB/IACUC for your proposal. If IRB or IACUC review is unavoidably delayed beyond the submission of the application, enter “Pending” on the Proposal Cover Page, and be advised that the certification must be received within 90 days after the due date for which the application is submitted.
 - c. Provide your TIN and CAGE numbers. Every U.S. institution that submits a proposal to a U.S. agency must provide their permanently-assigned Taxpayer Identification Number (TIN) and must register with the Department of Defense Central Contractor Registration (CCR) database for a permanently-assigned Commercial and Government Entity (CAGE) number. Reference the 2003 NRA Proposers Guidebook <http://www.hq.nasa.gov/office/procurement/nraguidebook/> for additional information. If you are unsure of your institution’s TIN number, please contact your institution’s Office of Sponsored Research to obtain the your

institution's Taxpayer Identification Number (TIN) or Employer Identification Number (EIN).

Click on "Continue."

- 8) The Team Member Page screen will appear, where you can add or remove team members. Every proposal must specify the critically important personnel who are expected to play a significant role in the execution of the proposed effort and their institution of employment. Categories of personnel to be included as Team Members are described in Appendix B, Section III, Part D(5) and in Section 1.4.2 in the 2003 NRA Proposers Guidebook (<http://www.hq.nasa.gov/office/procurement/nraguidebook/>)

You must include your authorizing official as a team member. When you complete and print the proposal cover page, you will see signature blocks both for yourself and your authorizing official. You are required to submit one original signed (by both you and your authorizing official) cover page with your proposal hardcopies.

IMPORTANT: If the team member is not listed in our database, please have them add themselves as a new user to the system. You may then add them to your team member list.

Select "Continue" if there are no other team members. To add a team member, highlight the role option on the selection list, type in first and last name and click on search. When the resulting set appears, choose the appropriate radio button and click on ADD to add the person to the proposal. After you are done, click on "Continue."

- 9) After continuing from the Team Member Page, the Proposal Options Page appears.
- 10) Please fill out the budget form by clicking on the "Budget" button, filling in project costs, and clicking "Continue." This will bring you to the Proposal Budget Review Page. Click "Continue" if the information is correct.
- 11) After verifying your budget information, you will be returned to the Proposal Options Page. Click the "Show/Print" button.
- 12) For detailed budget information, you must use Forms C and D, provided at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/nra_forms2.rtf Sample copies of Forms C and D are also available in Appendix F.

Form D must be filled out for each year of grant support requested.

These forms cannot be electronically submitted. Fill out the forms and attach them to your proposal.

- 13) At the page entitled Proposal Information Item List, click “Continue” to preview your Proposal Cover Page. Print the cover page from your Internet browser once you have reviewed the information. The cover page must be signed by both the Principal Investigator and the authorizing official and attached to the front of your proposal before submission of hard copies to NASA.

By signing and submitting the proposal identified on the cover sheet, the Authorizing Official of the proposing institution (or the individual investigator if there is no proposing institution): 1) certifies that the statements made in the proposal are true and complete to the best of his/her knowledge; 2) agrees to accept the obligations to comply with NASA Award terms and conditions if an award is made as a result of this proposal; 3) provides certification to the following that are reproduced in their entirety in Appendix D of this NRA: (i) Certification Regarding Debarment, Suspension and Other Responsibility matters, (ii) Certification Regarding Lobbying, and (iii) Certification of Compliance with the NASA Regulations Pursuant to Nondiscrimination in Federally Assisted Programs.

- 12) You may edit and resubmit your proposal cover page at any time before the submission deadline as indicated in the Summary and Supplemental Information Section of this NRA. Please note that once you submit a proposal cover page, it can only be edited, not deleted. For title, team member, budget or any other changes, please edit your existing proposal cover page and resubmit changes to avoid duplicate records.

D. Instructions for Preparation and Delivery of Proposals

All proposals submitted must include the completed cover page form as described in this Appendix. The name of the Principal Investigator should appear in the upper right hand corner of each page of the proposal except on the cover page form, where special places are provided for this information. Note that the proposal must specify the period of performance for the work described; periods of performance may be for any duration up to the maximum duration identified in the Announcement section of this NRA but should be suitable for the project proposed.

Please use the proposal submission checklist template form (Appendix F, Form A), located at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/nra_forms2.rtf, to assist you in assembling your proposal.

The proposal must include the following material, in this order:

- (1) Proposal Cover Page: Solicited Proposal Application, including certification of compliance with U.S. code (if applicable). One signed original required. Please see “Instructions for Preparing and Electronically Submitting a Proposal Cover Page” (Appendix B, Section III, Part C) for instructions on how to complete the proposal cover page information.

- (2) Transmittal Letter or Prefatory Material, if any (see Appendix E, “Instructions for Responding to NASA Research Announcements,” for details).
- (3) Proposal Title Page, with Notice of Restriction on Use and Disclosure of Proposal Information, if any (see Appendix E, “Instructions for Responding to NASA Research Announcements,” for details).
- (4) Project Description

The length of the Project Description section of the proposal cannot exceed 20 pages using regular (12 point) type. Text must be printed on one side only and should have the following margins: left = 1.5”; Right, top, bottom = 1.0”. Referenced figures must be included in the 20 pages of the Project Description. The Bibliography section is not considered part of the 20-page project description. Proposals that exceed the 20-page limit for the project description (22-page limit for revised proposals; see below) will not be reviewed. The proposal should contain sufficient detail to enable reviewers to make informed judgments about the overall merit of the proposed research and about the probability that the investigators will be able to accomplish their stated objectives with current resources and the resources requested. In addition, the proposal should clearly indicate the relationship between the proposed work and the research emphases defined in this Announcement. Reviewers are not required to consider information presented as appendices or to view and/or consider Web links in their evaluation of the proposal.

New applications where the investigator has received NASA funding in related fields from 1999 through 2002: Results and evidence of progress of the associated NASA supported research must be presented as part of the project description. See “Instructions for Responding to NASA Research Announcements” for details.

Revised applications (revisions of 1999 through 2002 submissions) must be so designated on the proposal cover page and explained in the project description. This explanation should be presented in a separate section of **no more than two pages at the beginning of the project description**, and is in addition to the 20 pages allowed for the project description. Related changes to the research plan should be highlighted in the body of the project description. Changes within the proposal may be highlighted by appropriate bracketing, indenting, or changing of typography. Clearly present any work done since the prior version was submitted. **Revised applications that do not address the criticisms in the previous review will be considered non-responsive and will be returned without review.** See “Instructions for Responding to NASA Research Announcements” for additional information.

(5) Management Approach

Each proposal must specify a single Principal Investigator who is responsible for carrying out the proposed project and coordinating the work of other personnel involved in the project. In proposals that designate several senior professionals as

key participants in the research project, the management approach section should define the roles and responsibilities of each participant and note the proportion of each individual's time to be devoted to the proposed research activity. The proposal must clearly and unambiguously state whether these key personnel have reviewed the proposal and endorsed their participation.

Investigators are strongly encouraged to identify only the most critically important personnel (Team Members) to aid in the execution of their proposals. Should such positions be necessary, Co-Investigators (Co-Is) may be identified who are critical for the successful completion of research through the contribution of unique expertise and/or capabilities, and who serve under the direction of the PI, regardless of whether or not they receive compensation under the award. Most NRAs require a Co-I to have a well-defined role in the research that is defined in the Management section of the proposal. Evidence of a Co-I's commitment to participate is often requested through a brief letter to be included with the proposal.

Co-Principal Investigators (Co-PIs) are not permitted with the sole exception when a non-U.S. Co-Investigator is proposed. This exception is described in the third subcategory below.

There are three subcategories of Co-Is that a proposal may identify, as appropriate:

- A Co-I may be designated as the *Science PI* for those cases where the proposing institution does not permit that individual to formally serve as the PI as defined above. In such a case, the Science PI will be understood by NASA to be in charge of the scientific direction of the proposed work, although the formally designated PI is still held responsible for the overall direction of the effort and use of funds.
- A Co-I may be designated as an *Institutional PI* when their institution is making a major contribution to a proposal submitted by a PI from another institution.
- A Co-I from a non-U.S. institution may be designated as a *Co-Principal Investigator* (Co-PI) should such a designation serve required administrative purposes in that Co-I's institution and/or for the procurement of funding by that Co-I from their sponsoring funding authority.

Additional Team Member category positions are often included in proposals as defined as follows:

A *Postdoctoral Associate* holds a Ph.D. or equivalent degree and is identified as a major participant in the execution of the proposed research. Such personnel may be identified by name or only by function in those cases where their recruitment depends on the successful selection of the proposal.

Other Professional is a description appropriate for personnel who support a proposal in a critical albeit intermittent manner, such as a consulting staff scientist or a key Project Engineer and/or Manager, who is not identified as a Co-I or Postdoctoral Associate.

A Graduate Student included in a proposal is working for a post-graduate degree and will support the proposed research under direction of the PI. Such a student may be identified by name or only by function in case their recruitment depends on the successful selection of the proposal.

A Collaborator is an unfunded position included in a proposal, whose participation is less critical than a Co-I, but who is committed to provide a specific contribution to the proposal.

(6) Personnel/Biographical Sketches (Appendix F, Form B)

The biographical sketch for each investigator must not exceed two pages. If the list of qualifications and publications exceeds two pages, select the most pertinent information (see “Instructions for Responding to NASA Research Announcements” for details). **You must use the biographical sketch form (Form B, Appendix F) located at:**

http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/nra_forms2.rtf

This form cannot be electronically submitted. Fill out Form B for each investigator and attach it to your proposal.

(7) Facilities and Equipment (see Appendix E, “Instructions for Responding to NASA Research Announcements,” for details).

(8) Special Matters (specific information on animal or human subjects protocol approval required, if applicable).

For proposals employing human subjects and/or animals, assurance of compliance with human subjects and/or animal care and use provisions is required on the Proposal Cover Page. In addition, the application must include a statement from the applicant institution certifying that the proposed work will meet all Federal and local human subjects requirements and/or animal care and use requirements.

Policies for the protection of human subjects in NASA sponsored research projects are described in NASA Management Instruction (NMI) 7100.8B (*Protection of Human Research Subjects*). Animal use and care requirements are described in the NASA Code of Federal Regulations (CFR) 1232 (*Care and Use of Animals in the Conduct of NASA Activities*). Both documents are available from the Office of Biological and Physical Research, Code UB, NASA Headquarters, Washington, DC 20546.

Additional Requirements for Research Employing Human Subjects

A letter signed by the Chair of the Institutional Review Board (IRB) identifying the proposal submitted to NASA by title and certifying approval of proposed human subjects protocols and procedures should be included with each copy of the proposal. IRB certifications for other research proposals or grants cannot be substituted (even if they employ the same protocols and procedures).

If IRB certification is pending on the proposal due date, select “pending” from the IRB/IACUC section menu on the Proposal Cover Page, and include with each copy of the proposal a letter signed by the IRB Chair identifying the proposal by title and indicating the status of the IRB review process at the time of submission. IRB certification must be received no later than 90 days after the proposal due date. An application lacking the required IRB certification 90 days after the proposal due date will be considered incomplete and may be returned to the applicant without review.

With regard to research involving human subjects, NASA and the NSBRI have adopted the National Institutes of Health (NIH) policy. Women and members of minority groups and their subpopulations must be included in NASA-supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided showing that inclusion of these groups is inappropriate with respect to the health of the subjects or the purpose of the research.

NASA will require current IRB certification prior to each year’s award.

Additional Requirements for Research Employing Animals

Specific information describing and justifying the use of animal subjects must be included in the proposal.

A letter signed by the Chair of the Institutional Animal Care and Use Committee (IACUC) identifying the proposal submitted to NASA by title and certifying approval of the proposed animal research protocols and procedures should be included with each copy of the proposal. The institution’s Public Health Service Animal Welfare Assurance Number must be included on the IACUC certification and entered in the IRB/IACUC section of the Proposal Cover Page. IACUC certifications for other research proposals or grants cannot be substituted (even if they employ the same protocols and procedures).

If IACUC certification is pending on the proposal due date, select “pending” from the IRB/IACUC selection menu on the Proposal Cover Page, and include with each copy of the proposal a letter signed by the IACUC Chair identifying the proposal by title and indicating the status of the IACUC review process at the time of submission. IACUC certification must be received no later than 90 days after the proposal due

date. An application lacking the required IACUC certification 90 days after the proposal due date will be considered incomplete and may be returned to the applicant without review.

NASA will require current IACUC certification prior to each year's award.

(9) Detailed Budget and Supporting Budgetary Information (Appendix F, Forms C and D).

For detailed budget information, you must use Forms C and D, provided at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/nra_forms2.rtf

Form D must be filled out for each year of grant support requested.

These forms cannot be electronically submitted. Fill out the forms and attach them to your proposal.

NASA is expected to be operating on the basis of full cost accounting as soon as possible, including all Civil Service salaries with overhead. In the interim period, proposals should use the accounting method authorized at their institutions at the time proposals are due and for the entire proposed period of performance. Funds to support the Resident Research Assistant (RRA) Postdoctoral Program costs (e.g., stipend, travel, computer time, supplies, etc.) are to be budgeted within the NASA intramural Principal Investigator budget.

If travel is planned, the proposal budget should include appropriate travel funds for visits to NASA field centers (as appropriate) and presentation of findings at professional society meetings.

In this solicitation, the terms "cost" and "budget" are used synonymously. Sufficient proposal cost detail and supporting information are required; funding amounts proposed with no explanation (e.g., Equipment: \$1,000, or Labor: \$6,000) may cause delays in evaluation and award. Generally, costs will be evaluated for realism, reasonableness, allowability, and allocation. The budgetary forms define the desired detail, but each category should be explained. Offerors should exercise prudent judgment in determining what to include in the proposal, as the amount of detail necessarily varies with the complexity of the proposal.

The following examples indicate the suggested method of preparing a cost breakdown:

Direct Labor

Labor costs should be segregated by titles or disciplines with estimated hours and rates for each. Estimates should include a basis of estimate, such as currently paid rates or outstanding offers to prospective employees. This format allows the

Government to assess cost reasonableness by various means including comparison to similar skills at other organizations.

Other Direct Costs

Please detail, explain, and substantiate other significant cost categories as described below:

- Subcontracts: Describe the work to be contracted, estimated amount, recipient (if known), and the reason for subcontracting.
- Consultants: Identify consultants to be used, why they are necessary, the time they will spend on the project, and the rates of pay.
- Equipment: List separately. Explain the need for items costing more than \$5,000. Describe basis for estimated cost. General-purpose equipment is not allowable as a direct cost unless specifically approved by the NASA Grant Officer. Any equipment purchase requested as a direct charge must include the equipment description, how it will be used in the conduct of the basic research proposed, and why it cannot be purchased with indirect funds.
- Supplies: Provide general categories of needed supplies, the method of acquisition, and estimated cost.
- Travel: Describe the purpose of the proposed travel in relation to the grant, and provide the basis of estimate, including information on destination and number of travelers (if known).
- Other: Enter the total of direct costs not covered by a) through e). Attach an itemized list explaining the need for each item and the basis for the estimate.

Indirect Costs

Indirect costs should be explained to an extent that will allow the Government to understand the basis for the estimate. Examples of prior year historical rates, current variances from those rates, or an explanation of other basis of estimates should be included. Where costs are based on allocation percentages or dollar rates, an explanation of rate and application base relationships should be given. For example, the base to which the General and Administrative (G&A) rate is applied could be explained as: application base equals total costs before G&A less subcontracts.

All awards made as a result of this NRA maybe funded as grants or contracts. However, while proposals submitted by “for profit” organizations are allowed, they cannot include a “fee.”

- (10) Other Support: You must complete Form E for specific sources of other support for the principal investigator and each Co-Investigator (not consultants). Form E is available at http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/nra_forms2.rtf
- (11) Appendices, if any (reviewers are not required to consider information presented in appendices).

- (12) One (1) signed original and twenty (20) copies of the proposal cover page and the proposals must be received by **5:00 p.m., July 15, 2003**, at the following address:

NASA Peer Review Services
SUBJECT: 03-OBPR-04 BR&C Ground-based Research Proposal
500 E Street SW, Suite 200
Washington, DC 20024
202-479-9030

IV. Compliance Matrix

The following information is specific to this NRA and **supersedes** the information contained in paragraphs (i) and (j) of "Instructions for Responding to NASA Research Announcements."

All proposals must comply with the general requirements of the Announcement as described in both Appendices A, B and Appendix E "Instructions for Responding to NASA Research Announcements." Appendices A and B contain specific requirements and explanations for each section of the proposal above and beyond the NASA-specified requirements. Appendix E, "Instructions for Responding to NASA Research Announcements," outlines the NASA-specified requirements for proposal submission and should be used for clarification and reference. Upon receipt, proposals will be reviewed for compliance with the requirements of this Announcement. This includes the following:

1. Submission of complete proposals specified in this Announcement. Proposals must be responsive to the areas of program element emphasis described in this Announcement and include a project description that is not more than 20 pages in length.
2. Submission of appropriate Institutional Review Board (IRB) or Animal Care and Use Committee (ACUC) certification for all proposals using human or animal test subjects.
3. Submission of a budget that is within the guidelines specified in this Announcement and is for a funding period not exceeding that described in the Announcement.
4. Proposals that are revised versions of proposals previously submitted to NASA must be clearly designated as such on the proposal cover page and must contain an explanation of how the revised proposal has addressed criticisms from previous NASA review. This explanation should be presented in a separate section of no more than two pages at the beginning of the project description and is in addition to the 20 pages allowed for the project description. Related changes to the research plan should be highlighted in the body of the project description.
5. Submission of all other appropriate information as required by this NASA Research Announcement (refer to Appendices A and B).

Note: At NASA's discretion, non-compliant proposals may be withdrawn from the review process and returned to the investigator without further review.

Compliant proposals submitted in response to this Announcement will undergo an intrinsic scientific or technical merit review. Only those proposals most highly rated in the merit review process will undergo additional reviews for program relevance and cost.

V. Eligibility

All categories of institutions are eligible to submit proposals in response to this NRA, but only approved proposals from U.S. institutions will be selected for funding. Principal Investigators may collaborate with universities, Federal Government laboratories, the private sector, and state and local government laboratories. In all such arrangements, the applying entity is expected to be responsible for administering the project according to the management approach presented in the proposal.

The applying entity must have in place a documented base of ongoing high quality research in science and technology or in those areas of science and engineering clearly relevant to the specific programmatic objectives and research emphases indicated in this Announcement. Present or prior support by NASA of research or training in any institution or for any investigator is neither a prerequisite to submission of a proposal nor a competing factor in the selection process.

VI. Guidelines for International Participation

Guidelines for International Participation are detailed in paragraph (I) of Appendix E of this Announcement.

Export Control Guidelines Applicable to Foreign Proposals and Proposals Including Foreign Participation. Foreign proposals and proposals including foreign participation must include a section discussing compliance with U.S. export laws and regulations, e.g., 22 CFR Parts 120-130 and 15 CFR Parts 730-774, as applicable to the circumstances surrounding the particular foreign participation. The discussion must describe in detail the proposed foreign participation and is to include, but not be limited to, whether or not the foreign participation may require the prospective investigator to obtain the prior approval of the Department of State or the Department of Commerce via a technical assistance agreement or an export license, or whether a license exemption/exception may apply. If prior approvals via licenses are necessary, discuss whether the license has been applied for or if not, the projected timing of the application and any implications for the schedule. Information regarding U.S. export regulations is available at <http://www.pmdtc.org/> and <http://www.bxa.doc.gov/>. Investigators are advised that under U.S. law and regulations, spacecraft and their specifically designed, modified, or configured systems, components, and parts are generally considered “Defense Articles” on the United States Munitions List and are subject to the provisions of the International Traffic in Arms Regulations (ITAR), 22 CFR Parts 120-130.

**NASA Research Announcement
Ground-based Research Proposals**

Biomedical Research and Countermeasures Program

**Research Projects for a Research Team of the National Space Biomedical
Research Institute**

NOTE 1: The National Space Biomedical Research Institute (NSBRI) is soliciting integrative, cross-disciplinary proposals dealing with the effects of space radiation on a variety of aspects of human functioning in space. The Institute will not, however, be accepting proposals for the Radiation Effects Team. Proposals that synergistically bridge multiple disciplines for the purpose of modeling the effects of microgravity on the human body to aid in the development and testing of countermeasures, or to develop technologies that enable research in one or more NSBRI research areas are strongly encouraged. Applications that incorporate innovative bioinformatics approaches to acquisition and assessment of biomedical data are also invited.

NOTE 2: The overall focus for NSBRI proposals must be the definition and feasibility of specific practical countermeasures (CRLs 3-7).

NOTE 3: Only investigators who are applying to join one of the NSBRI research teams listed in Section I should use this appendix for the preparation of their application. Scientists interested in submitting proposals for independent investigator research projects should refer to Appendix B.

I. Introduction

The NSBRI is a private, non-profit organization competitively selected by NASA. The mission of the Institute is to use an integrated research team approach to advance biomedical research with the goal of ensuring safe and productive long-term human exploration of space. The NSBRI invites ground-based research applications to join an existing team in one of 10 research areas:

1. *Bone Loss* – Addressing bone loss and weakening during space flight, and the inherent fracture risks.
2. *Cardiovascular Alterations* – Addressing the in-flight occurrence of cardiac dysrhythmias and post-flight impairment of the cardiovascular response to orthostatic and exercise stress.

3. *Human Performance Factors, Sleep, and Chronobiology* – Investigating maintenance of high cognitive performance and vigilance despite environmental stress and sleep disturbances.
4. *Immunology, Infection, and Hematology* – Addressing immune system impairment and altered susceptibility to infection, increased allergic responsiveness, decreased blood volume, and post-flight anemia.
5. *Muscle Alterations and Atrophy* – Focusing on the loss of skeletal muscle mass, strength, and endurance that accompanies space flight.
6. *Neurobehavioral and Psychosocial Factors* – Investigating methods and tools that can be utilized to enable crews to cope with stress, isolation, and compatibility.
7. *Neurovestibular Adaptation* – Addressing the problems of space motion sickness and disorientation during flight and the post-flight problems of balance and gaze disorders.
8. *Nutrition, Physical Fitness, and Rehabilitation* – Developing methods to maintain health and fitness before, during, and after space flights.
9. *Smart Medical Systems* – Developing new methods of non-invasive medical monitoring, diagnosis, and therapy for use on space missions.
10. *Technology Development* – Developing instrumentation and other technological products that will enhance the research of the other teams and benefit people on Earth.

Each of the ten research teams consists of a set of coordinated and complementary projects focused on a common theme. Team management and coordination is the responsibility of the **Team Leader**. A single Team Leader, assisted by an Associate Team Leader, heads each research team. Team Leaders play a pivotal role in guiding the Institute's research program and in the ultimate success of the Institute. Their expertise and "hands-on" approach to research management add value across projects and across teams. The Team Leader is guided by the Critical Path Roadmap (CPR), which is the cornerstone for developing the team's integrated strategic research plan, the key to accomplishing the Institute's mission.

Team Leader positions for all ten teams will be competed in parallel with this Announcement. See the Call for Candidates for more information on how to apply for these leadership positions at <http://www.nsbri.org/Announcements/callforcandidates.html>

Research proposals will be accepted from all categories of organizations, public and private, and for-profit and non-profit, such as universities, colleges, hospitals, laboratories, units of state and local governments, and eligible agencies of the Federal Government. The mechanism of support shall be an NSBRI sub-agreement with funds provided by NASA through a cooperative agreement (Cooperative Agreement NCC 9-58) with NASA's Lyndon B. Johnson Space Center. Progress of the funded research will be reviewed annually. *Potential foreign applicants should note that, normally, the country of origin, not the NSBRI, must fund applications from non-U.S. organizations. Potential foreign applicants should coordinate their application with both the NSBRI and the appropriate funding agency in their own country.*

II. Background

The NSBRI is responsible for the development of countermeasures against the deleterious effects of long-duration space flight and applied space biomedical research directed toward this specific

goal. Its mission is to lead a national effort in integrated, critical path space biomedical research that supports NASA's Bioastronautics Strategy by focusing on the enabling of long-term human presence in, development of, and exploration of space. This is accomplished by:

- designing and testing effective countermeasures to address the biological and environmental impediments to long-term human space flight;
- defining the molecular, cellular, organ-level, and integrated responses and mechanistic relationships that ultimately determine these impediments, where such activity is essential for the development of novel countermeasures;
- establishing biomedical support technologies to maximize human performance in space, reducing biomedical hazards to an acceptable level, and delivering quality medical care;
- transferring and disseminating the biomedical advances in knowledge and technology to the general benefit of mankind; and
- ensuring open involvement of a diverse scientific community, industry, and the public at large in the Institute's activities and fostering a robust partnership with NASA, particularly through NASA's Lyndon B. Johnson Space Center.

Institute Infrastructure

The NSBRI is governed by a consortium of twelve institutions: Baylor College of Medicine; Brookhaven National Laboratory; Harvard Medical School; The Johns Hopkins University School of Medicine and the Applied Physics Laboratory; Massachusetts Institute of Technology; Morehouse School of Medicine; Mount Sinai School of Medicine; Rice University; Texas A&M University; the University of Arkansas for Medical Sciences; the University of Pennsylvania Health System; and the University of Washington. The Institute's Headquarters are located in Houston, at Baylor College of Medicine.

This is an open solicitation. Consortium membership is not a requirement for research program participation. The management plan for the Institute is based on the model used by the National Institutes of Health. An independent Board of Scientific Counselors (BSC) is responsible for assuring excellence in the Institute's research program through independent external peer review. An External Advisory Council (EAC) is responsible for advising Institute management and the Board of Directors (comprised of, but not limited to, representatives from the senior management of each of the 12 NSBRI Consortium member institutions) concerning program strategy, tactical implementation, and effectiveness. The NSBRI also has a User Panel of former and current astronauts and flight surgeons responsible for assuring that the research program is focused squarely on astronaut health and safety. An Industry Forum of representatives from the space and biomedically-related industries advises and assists the NSBRI concerning Earth- and space-based applications for Institute research. In addition to its research program, the NSBRI has developed a vital education and outreach program that takes advantage of the Institute's core research activities. The Institute coordinates its research activities with NASA through a joint NASA/NSBRI Steering Committee.

Call for Candidates – Team Leaders

As described in the NSBRI Policy on Team Leadership

(<http://www.nsbri.org/Announcements/callforcandidates.html>), Team Leaders are selected by the NSBRI Director with the approval of the NSBRI Board of Directors for a term that is identical with the term of their NSBRI-funded research project. The NSBRI is soliciting applications for team leaders on a competitive basis. Current Team Leaders may reapply for the next term. **Prospective Team Leaders must prepare and submit in response to this solicitation, as Principal Investigator, a proposal that achieves a merit score in the competitive range as a prerequisite for being considered for a Team Leadership position.** All applications for Team Leader positions will be considered new applications. No special consideration will be given to current Team Leaders who reapply. A separate application process will be used to select Team Leaders. **Do not make any reference to your interest in becoming a Team Leader in your NSBRI proposal application.**

For more information about applying for a Team Leadership position, go to the Call for Candidates at <http://www.nsbri.org/Announcements/callforcandidates.html>.

III. Specific Research Focus and Opportunity

General Information

To carry out the NSBRI's primary mission of identifying, designing and developing effective countermeasures to address the biological and environmental impediments to human space flight, the NSBRI focuses its research program on the primary needs of long-duration missions (e.g., several months on the International Space Station, exploration class missions, etc.). These missions pose the greatest challenge to present and future space travelers, and meeting these challenges with appropriate countermeasures lies at the core of the NSBRI's responsibility.

Potential physiological changes that may occur during prolonged space flight include, among others, significant loss of muscle and bone mass, decreased dietary intake of nutrients, profound metabolic and endocrine alterations, important changes in cardiovascular function, and deleterious effects on sensorimotor performance. By addressing long-term missions, increased crew safety, health, and performance will be realized for shorter-duration space flights.

NSBRI research is conducted in partnership with NASA using an **integrated team approach**. The teams focus on high priority biomedical research problems and investigators work together, within and between teams, to address complex risks that often require interdisciplinary expertise and resources. The value added in the integrated team approach leads to more effective outcome-driven research than what is obtainable by a single project alone.

The NSBRI has an essential enabling role for NASA: providing capabilities for countermeasures development research. The Institute engages scientists, engineers, and clinicians and utilizes the resources of institutions to form a biomedical research community. Countermeasures research conducted by the NSBRI integrates a biomedical research community with the engineering and operational expertise of NASA to effectively manage health risks for long-duration human space flight.

The NSBRI's research program contains studies that, for the most part, range from CRL 3 through 7. Each proposer must examine and understand the CRL scale and specify in the proposal the CRL that will result from the funding and conduct of the proposed research. For further information, refer to Appendix A.

For more information on the NSBRI, see <http://www.nsbri.org/>.

NSBRI Team Specific Research Focus and Opportunity

Proposals submitted to the NSBRI in response to this NRA must address one of the ten research areas discussed below. Proposals that impact more than one area should be directed to only one primary research area, although a secondary research area may be designated if the proposal has significant overlap with that area. Studies that use integrated methods are particularly encouraged.

It is recommended that investigators carefully review the Team Strategic Plan of the team(s) relevant to a proposal. These plans are referenced in the following subsections, which are meant to guide the investigator to the key problems and issues that are central to each research area. In all cases, proposals must represent questions and be relevant to priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>.

Proposals in radiation, modeling, space medicine, and technology that are relevant to countermeasure development within the scope of the NSBRI mission are invited but must address one of the 10 research areas discussed below. The NSBRI seeks innovative projects of varying duration (up to four years) and scope that will produce clear deliverables in the CRL 3-7 range. Proposers are encouraged to define clear milestones and to collaborate with NASA scientists, engineers, flight surgeons, and astronauts, as appropriate, to maximize the likelihood of success and impact of their proposed research.

1. NSBRI Bone Loss Team

The Bone Loss Team studies the mechanisms involved in bone loss related to microgravity, the development of countermeasures to prevent bone loss, and methods for evaluating the rate of loss and the impact on fracture risk. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Bone.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- What pharmacological, nutritional, or mechanical treatments, or combinations thereof, effectively diminish the loss of bone mass in weightless or non-weight bearing conditions that simulate microgravity? Investigation of this question may require chronic bed rest studies.
- Which factors (genetic, baseline values, fitness, etc.) determine the wide variation in inter-individual rates and site-specific patterns of bone loss?
- Which radiological or imaging methods best permit geometric or structural analysis of patterns and rates of bone loss in humans subjected to microgravity?

- Is there a means of accelerating the recovery of bone following exposure to weightlessness? Does the delayed return of bone mass to pre-flight levels increase injury risk during rehabilitation? If so, how can bone recovery be accelerated?
- What is the nature and incidence rate of soft connective tissue injury and related symptoms during and after prolonged space flight?
- Can one quantify the incidence or extent of injury to intervertebral discs during weightlessness or upon return to normal gravity?
- Which procedures will protect against soft tissue injury in-flight and hasten repair of damaged soft tissues?
- Can alterations in the timing and consolidation of fracture callus that forms during disuse/microgravity be normalized, and with what pharmacological or mechanical interventions?
- What modalities are practical for space flight applications that might accelerate fracture healing?
- Can fracture risk be accurately predicted from novel modeling techniques developed from available bone loss data collected on astronauts/cosmonauts?
- The development of novel nutritional and pharmacological countermeasures to reduce renal stone formation.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

2. NSBRI Cardiovascular Alterations Team

The Cardiovascular Alterations Team is focused on understanding the mechanisms of and identifying effective solutions to conditions wherein astronauts may experience heart rhythm disturbances, cardiac atrophy, and a drop in blood pressure, causing faintness, reduced exercise capacity, and decreased function following landing. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Cardio.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- Countermeasures to reduce impaired cardiovascular responses to orthostatic stress.
- Occurrence of serious cardiac dysrhythmias and methods to predict and prevent such events.
- Cardiac atrophy and remodeling.
- Techniques to address the manifestation of previously asymptomatic cardiovascular disease that may present during space missions.
- Impaired cardiovascular response to exercise stress.
- Development of new cardiovascular technologies for space flight and Earth based applications.
- Individual susceptibility to the adverse effects of space flight on the cardiovascular system.
- Strategies for short-term and long-term cardiovascular rehabilitation following space flight.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

3. NSBRI Human Performance Factors, Sleep, and Chronobiology Team

The Human Performance Factors, Sleep, and Chronobiology Team is developing ways to reduce human mistakes and optimize mental and physical performance during long-duration space flight. The loss of 24-hour day/light cycle, weightlessness, a confined environment, and work demands make sleep difficult in space. Cumulative sleep loss increases the risk of accidents and possible mission failure. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Sleep.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- Which photic, behavioral, environmental, pharmacological, nutritional, and/or exercise countermeasures will help crew members reduce disturbances of circadian rhythmicity, sleep disturbances, or homeostatic sleep drive, thereby reducing the associated performance deficits?
- How can performance during prolonged space flight be optimized by manipulating the neurobiological processes underlying sleep and/or circadian rhythmicity?
- What are the most sensitive and specific methods for monitoring the ongoing status of sleep, sleep homeostasis, circadian regulation, and individual light exposure, performance capability, metabolic functions, and physical health during extended duration space flight?
- What are the best optimization techniques for using mathematical models of sleep homeostasis and circadian regulation to specify and schedule countermeasure strategies?
- What measures of sleep, sleep disorders, or circadian function predict individual neurobehavioral performance, adaptation, metabolic function, physical health, or countermeasure efficacy?
- What are the effects of space flight on the pharmacokinetics, efficacy, side effects, and interactions (drug-drug, drug-sleep, drug-circadian) of therapeutic agents designed to improve sleep, circadian regulation, cognitive performance, and physical health?
- What technological and procedural advances can minimize the probability of error by astronauts whose abilities may be impaired by fatigue or circadian disruption?
- How can advances in computer-aided decision making, on-board training, or smart check lists be applied to offset cognitive deficits?
- How can recent advances in the neurobiology of sleep and/or circadian rhythms (e.g., orexin/hypocretin system, circadian photoreception, output pathways for regulation of sleep or circadian rhythms, peripheral oscillators) be used to develop countermeasures to facilitate adaptation to the space environment and thereby maintain optimal neurobehavioral performance during an exploration-class space mission?
- How do countermeasures intended for other physiologic systems (e.g., exercise, activity schedules) interact with sleep, circadian organization, and waking function in long

duration space flight? How might the timing of such countermeasure administration be used to improve sleep, circadian organization, waking performance, or physical health?

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

4. NSBRI Immunology, Infection, and Hematology Team

The Immunology, Infection, and Hematology Team is examining the effects that extended space flight might have on virus reactivation and a weakened immune system. Radiation damage to the bone marrow stem cell raises concern of space flight-related anemia and other blood cell deficiencies following a mission. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Immune.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- Effect of space environmental conditions on long-term risks of viral-induced malignancies.
- Countermeasures to address radiation effects on host control of infection: mucosal vs. systemic immunity.
- Effects of microgravity and/or radiation on virulence of microbes and on the host: microorganism homeostasis.
- Assessment of space station vs. deep space radiation effects on bone marrow.
- Pleuripotent stem cells and hematopoietic progenitor cells: iron, silicon.
- Effects of space radiation on T-cell function.
- Mechanisms of transmission of microbial agents in space flight conditions.
- Role of defensins in wound-healing in space.
- Development of monitoring systems for microorganisms and virus reactivation.
- Stem cell reconstitution using an irradiated mouse model.
- Mechanisms to resist infections and malignancies in space: nutritional supplements, hormones, antibodies, pharmaceuticals, and vaccines.
- Use of gene inserts to develop resistance to radiation-induced pathogens.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

5. NSBRI Muscle Alterations and Atrophy Team

The Muscle Alterations and Atrophy Team's objective is to develop methods to prevent or reduce muscle loss on space missions. While astronauts exercise in space, current exercise regiments alone are not sufficient to prevent potentially deleterious changes that occur in skeletal

muscle during space flight. The Team works to identify effective physical countermeasures (i.e., exercise prescriptions) and to combine this strategy with other countermeasures, such as improved nutrition and pharmacological interventions. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Muscle.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- Effects of resistance training as a countermeasure to muscle alterations and atrophy in simulated microgravity (e.g. altered loading states).
- Are there synergistic effects when various activity paradigms are carried out simultaneously with other countermeasures, such as nutritional modification and/or pharmacological intervention and other strategies, such as antioxidants and vitamin supplements?
- Is it necessary to maintain or regain muscle mass in order to maintain muscle strength and power generating capacity?
- Are there practical programs predicated on activity paradigms that can maintain the normal phenotypes typically seen in the muscles of humans and animals?
- How do altered loading states affect the sensory motor processes that affect posture, balance, and the performance of locomotor tasks of varying intensity and complexity?
- What are the stress/strain reactions that impact force production, and do muscle atrophy and injury processes affect these properties?
- Are atrophying skeletal muscle, the myotendinous junctions, tendons, and ligaments more prone to injury and are the mechanisms of recovery from injury altered?
- How does artificial gravity (e.g., gravity-equivalent acceleration and variable-G forces) affect the structure and function of human skeletal muscle in normal and atrophying skeletal muscle?
- Are there systems other than skeletal muscle that are impacted by artificial gravity?
- Can artificial gravity approaches interact with other paradigms impacting high stress levels on the musculoskeletal system?

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

6. NSBRI Neurobehavioral and Psychosocial Factors Team

The Neurobehavioral and Psychosocial Factors Team is concerned with methods crews use to deal with stress, isolation, confinement, and the challenges of long duration space missions. In addition to identifying neurobehavioral and psychosocial risks to crew health, safety, and productivity, team objectives include developing methods to monitor brain functions and behavior and countermeasures to enhance performance, motivation, and quality of life. Leadership style, crew composition, organization, and communication are also being investigated to optimize crew effectiveness and mission success. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Psycho.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- What are the effects of culture, gender, personality, leadership, and training on performance, stress, and health in isolated groups in confined environments and ground-based, analog environments for space flight?
- What are the major influences on interpersonal actions, communications, and problem solving in small isolated groups and what techniques can be developed to optimize group dynamics and performance?
- How can affective, neurobehavioral, and neurocognitive dysfunction be objectively detected in remote locations?
- What objective, unobtrusive methods and approaches will permit detection of stress, declining cognitive, emotional, and social functions, and changes in operationally relevant performance capabilities during space flight?
- What are the effects of space radiation on cognitive and other brain functions, and what countermeasure strategies should be developed to minimize the potential harmful central nervous system effects of radiation exposure?
- What neurobiological processes of stress and arousal are the optimal targets for behavioral and pharmacological interventions?
- What behavioral and pharmacological interventions are optimal in space flight?
- What are the effects of long-term exposure to the major factors in the space environment on emotions (including emotional reactivity, stress neurobiology and responses, modulation of mood, and vulnerability to affective disorders), cognition and performance (including processes of sensation and perception, learning, vigilance, problem solving, decision making, and motor skills), and behavioral health?
- What are the behavioral strategies, scheduling and timeline approaches, and habitability design elements that can maintain or enhance crew performance and prevent the development of hostility within flight crews and between crews and ground-support personnel during long-duration space flight?
- How can mathematical models of human interaction and the temporal dynamics of human behavior help predict responses in space flight?

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

7. NSBRI Neurovestibular Adaptation Team

The Neurovestibular Adaptation Team is developing potential preflight and in-flight countermeasures to allow crew members to adjust more rapidly to gravitational changes that can result in disorientation, motion sickness and a loss of sense of direction. These problems have an impact on space motion sickness, landing and post-flight adaptation. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Neuro.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- What causes the profound impairments of posture, gaze, visual acuity, ataxia, and locomotion stability in astronauts, and what countermeasures minimize these impairments to reduce re-entry and landing vertigo, acute space motion sickness, post-flight imbalance, and in-flight spatial orientation?
- Are some crew members more susceptible to re-entry and landing vertigo than others and does repeated microgravity experience confer significant immunity?
- Can new, safe, and effective anti-motion sickness drugs be developed which specifically target emetic centers or the vestibular-emetic linkage, act rapidly, and do not impair cognition or performance?
- Can improved anti-motion sickness delivery systems and dose and side effect monitoring systems be developed, and what are the best ground-based methods for assessing the effectiveness and side effects of drug countermeasures and for evaluating microgravity pharmacokinetics?
- Are there non-pharmacologic techniques (e.g., parabolic flight preadaptation, head movement restriction) which could significantly reduce the incidence of acute space motion sickness, or which could mitigate the impact of emesis on EVA life support systems?
- What is the effect of cardiovascular, muscle, and skeletal rehabilitation therapies on neurovestibular recovery, and the converse?
- Can preflight or inflight training, balance exercises, sensory aids, prostheses, and assessment techniques improve postlanding postural and locomotor control and functional task performance?
- What spacecraft architectures and interior visual cues minimize disorientation?
- What are the effects of artificial gravity on human orientation and eye, head, and limb movements, and what are the pros and cons of various types of artificial gravity as countermeasures against the effects of microgravity on neurovestibular function?
- Does long-term exposure to microgravity or partial gravity, radiation, or environmental toxins cause functionally important, irreversible (pathophysiological) changes in central or peripheral vestibular and sensorimotor function, development, or plasticity, or cause acceleration of the normal aging process?
- Does microgravity-altered calcium homeostasis impact otoconial formation, and if there are important effects, what countermeasures are appropriate?

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

8. NSBRI Nutrition, Physical Fitness and Rehabilitation Team

The Nutrition, Physical Fitness, and Rehabilitation Team is addressing the quality and quantity of dietary intake, exercise, and rehabilitation to reduce or eliminate muscle atrophy and bone loss, and to improve altered cardiovascular function. The Team is also examining countermeasures to reduce the biomedical risks of radiation, circadian alterations, and other factors associated with long duration human space missions. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and

priorities, are located at <http://www.nsbri.org/Research/Nutrition.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas and questions:

- Understanding mechanisms and designing effective nutritional countermeasures to the deficiencies in thirst and nutrient intake, with relevance to changes that may occur during human space flight.
- Establishing ground-based clinical measurements of biochemical alterations that may indicate depression of food intake.
- Developing monitoring methods for assessment of food intake and physical activity that are relevant to the space environment.
- Nutrition and/or physical fitness countermeasures to high priority problems, with emphasis on radiation-enhanced carcinogenesis, depression of cognitive function, bone loss, and loss of muscle mass and function.
- Studies that assess the effectiveness of aerobic and resistive exercise countermeasures, with endpoint parameters that quantify the cardiovascular response, bone metabolism, body composition, and skeletal muscle metabolism and function.
- Assessment of exercise countermeasures that include strict dietary control and contain measures of energy balance.
- Development of accurate methods to assess body composition changes relevant to human space flight.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

9. NSBRI Smart Medical Systems Team

The Smart Medical Systems Team is developing and applying new technologies for physiological and medical monitoring and clinical care that integrate novel hardware, intelligent algorithms and models, and new therapeutic approaches applicable for remote health care in the space environment and on Earth. The Team works closely with the Technology Development Team and the Space Medicine group at Johnson Space Center. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at http://www.nsbri.org/Research/Med_Sys.html.

Proposals are sought whose research addresses, but is not limited to, the following areas:

- Novel sensor systems for remote physiological monitoring and medical diagnosis.
- Novel diagnostic and therapeutic hardware modalities to reduce risk and problems associated with trauma and acute medical conditions that might occur in the space environment.
- Innovative imaging strategies with automated, intelligent diagnostic interpretation capabilities.
- Methods to reduce risk of and manage toxic exposure in a space environment.

- Methods to better understand and reduce risk of altered pharmacodynamics, adverse drug reactions, and drug interactions.
- Decision support systems and knowledge bases for diagnosis and treatment that interface humans and machines, and enhance clinical care and medical training for crew medical officers and flight surgeons.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

10. NSBRI Technology Development Team

The Technology Development Team develops new devices and systems to improve research techniques for the other teams, and adds value to the enabling scientific and medical technologies already supported by the other teams and by NASA. Projects focus on designing lightweight, compact research tools and on developing simple, minimally-invasive and non-invasive methods of gathering health-related data that are relevant to space missions and have Earth-based applications. Team information and the Team Strategic Plan for countermeasures research and development, including research goals and priorities, are located at <http://www.nsbri.org/Research/Tech.html>.

Proposals are sought whose research addresses, but is not limited to, the following areas:

- Development of multi-purpose instruments or devices to monitor physiological measures (e.g., vital signs, core body temperature, eye motion, body fluid chemistry, etc.) using sensors and sensor systems that are easy to use, non-invasive (or minimally invasive), comfortable to wear, unobtrusive, and non-interfering with task performance.
- Innovative technologies applicable to the space environment to detect and identify pathogens (including bacteria, fungi, and viruses) in air, water samples, food, and human specimens, utilizing small sample volumes, fast read-out, and automated methods.
- Development of automated approaches to carrying out biochemical assays (especially in-flight) with minimal operator intervention.
- Development of novel devices to collect blood and other bodily fluids with minimum crew disturbance and discomfort.
- Advanced cabin communications and information management systems, including wireless and infrared optical systems, to facilitate the collection and analysis of important biological information without tethering or otherwise hampering astronaut activities.
- Low mass, compact diagnostic and therapeutic tools and equipment that use minimum spacecraft resources and augment the efforts of the NSBRI Smart Medical Systems Team and NASA Space Medicine to enrich the in-flight clinical status evaluation of crews.

Proposals must represent questions and priorities enumerated in the CPR at <http://criticalpath.jsc.nasa.gov/>. Investigators must complete Form F in Appendix F for consideration of the proposal. **Form F can be found in the downloadable documents section of the on-line proposal submission system. After completion, Form F should be uploaded as an Appendix in the on-line proposal.**

IV. Application Procedures for the Opportunity to Participate on a National Space Biomedical Research Institute Team

Proposals to join an NSBRI research team must comply with the requirements of this research opportunity as described in this appendix (Appendix C). Appendix D outlines general NASA-specified requirements for proposal submission and should be used only for clarification of matters not specifically discussed here. Appendix C supersedes, modifies, or extends the requirements enumerated in Appendix D.

General Instructions

Proposals to join one of the NSBRI's ten research teams must be submitted through NSBRI's Internet-based Electronic Proposal Submission System (EPSS). Applications for NSBRI Team Leadership will be handled separately from this solicitation. Please go to the Call for Candidates at <http://www.nsbri.org/Announcements/callforcandidates.html> for information on applying for Team Leadership positions.

The EPSS has been designed to enable investigators to collaborate on the development of a proposal, to retain complete privacy throughout the proposal development process, and to allow fast and accurate proposal submission. If a proposal is selected for funding, the electronic proposal information will serve as an active record file, enabling simplified investigator information changes, annual report submission, and NASA Task Book submission.

The Notice of Intent to propose is prepared and electronically submitted through EPSS. To assure that the notice of intent is submitted by **May 15, 2003**, go to the Web site <http://myportal.nsbri.org/> and register to obtain a personal account on the system. After entering contact information, investigators will receive a username and password for entry into EPSS and can enter the limited information required for a notice of intent. After this, the above Web address will serve as the entry point for proposal development and modification. All information entered, with the exception of that required for the notice of intent, will remain private until electronic submission is completed.

Proposal information requested in EPSS closely follows the information requested by NIH grant application form PHS 398. This information includes Basic Personal and Institutional Information, Project Description, Performance Sites, Key Personnel, Investigator Budgets with Justifications, Other Support, Biographical Sketches, Laboratory Resources, and Research Plan.

A proposal overview screen will guide applicants through the process of completing the required proposal information. EPSS offers a collaborative work environment for the Principal Investigator and Co-Investigators to view and submit various portions of the proposal. For example, the Principal Investigator can enter or upload all information for the proposal. Co-Investigators can view most of the proposal information but are permitted to enter only their specific personal information and their assigned project and budgetary information. All investigators can allow an administrative support person to act on their behalf, to assist in the entry of proposal information; however, electronic submission can only be performed by the Principal Investigator. EPSS will contain an Investigator Profile section, containing biographical sketches and other information, for each investigator registered in the system. This information

can be used by authorized proposing investigators, eliminating the duplicate entry of such information.

Electronic proposals and applications must be submitted before 5:00 p.m. EST, Tuesday, July 15, 2003. After submission using EPSS, the Principal Investigator **must** mail the printed proposal cover page that is generated by the system, with the appropriate institutional approvals, to the following address within **one week** of the submission deadline:

NSBRI, *Attn: NRA 03-OBPR-04*
One Baylor Plaza, NA-425
Houston, TX 77030-3498
713-798-7412

Please direct any questions concerning this application procedure to the NSBRI by calling 713-798-7412, by faxing your questions to 713-798-7413, or by sending your inquiry to contact_us@www.nsbri.org. The technical requirements to operate EPSS are Internet Explorer 4.0+ or Netscape 4.03+ for Windows, Macintosh, or Unix. EPSS is best viewed using Internet Explorer 6.0.

Eligibility – All categories of institutions are eligible to submit proposals in response to this NRA, but, in most cases, only approved proposals from U.S. institutions will be selected for funding. Principal Investigators may collaborate with universities, Federal Government laboratories, the private sector, and state and local government laboratories. In all such arrangements, the applying entity is expected to be responsible for administering the project according to the management approach presented in the proposal.

The applying entity must have in place a documented base of ongoing high quality research in science and technology or in those areas of science and engineering clearly relevant to the specific programmatic objectives and research emphases indicated in this Announcement. Present or prior support by NASA or the NSBRI of research or training in any institution or for any investigator is neither a prerequisite to submission of a proposal nor a competing factor in the selection process.

Notice of Intent – To facilitate planning for the review process, investigators are requested to submit a notice of intent to propose by using EPSS and following the online instructions. This non-binding notification should be submitted electronically by May 15, 2003.

Budgetary Matters – Budgets are to be prepared according to the instructions provided online through EPSS. It is expected that the average annual total (direct + indirect) cost of selected proposals will be approximately \$250,000. In general, the annual total cost of a single proposal should not exceed \$450,000. ***NSBRI awards require a cost-sharing arrangement with the institution consisting of an augmentation of at least 10% in addition to the total NSBRI award. This contribution should not be identified in the submitted project budget but will be requested at the time the institutional award is made.***

Special Matters – (specific information on animal or human subjects protocol approval required, if applicable).

For proposals employing human subjects and/or animals, assurance of compliance with human subjects and/or animal care and use provisions is required on the Proposal Cover Page. In addition, the application must include a statement from the applicant institution certifying that the proposed work will meet all Federal and local human subjects requirements and/or animal care and use requirements.

Policies for the protection of human subjects in NASA sponsored research projects are described in NASA Management Instruction (NMI) 7100.8B (*Protection of Human Research Subjects*). Animal use and care requirements are described in the NASA Code of Federal Regulations (CFR) 1232 (*Care and Use of Animals in the Conduct of NASA Activities*). Both documents are available from the Office of Biological and Physical Research, Code UB, NASA Headquarters, Washington, DC 20546.

Additional Requirements for Research Employing Human Subjects

A letter signed by the Chair of the Institutional Review Board (IRB) identifying the proposal submitted to NASA by title and certifying approval of proposed human subjects protocols and procedures should be included in the appendix of the proposal. IRB certifications for other research proposals or grants cannot be substituted (even if they employ the same protocols and procedures).

If IRB certification is pending on the proposal due date, select “pending” from the IRB/IACUC section menu on the Proposal Cover Page, and include in the appendix of the proposal a letter signed by the IRB Chair identifying the proposal by title and indicating the status of the IRB review process at the time of submission. IRB certification must be received no later than 90 days after the proposal due date. An application lacking the required IRB certification 90 days after the proposal due date will be considered incomplete and may be returned to the applicant without review.

With regard to research involving human subjects, NASA and the NSBRI have adopted the National Institutes of Health (NIH) policy. Women and members of minority groups and their subpopulations must be included in NASA-supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided showing that inclusion of these groups is inappropriate with respect to the health of the subjects or the purpose of the research.

NSBRI will require current IRB certification prior to each year’s award.

Additional Requirements for Research Employing Animals

Specific information describing and justifying the use of animal subjects must be included in the proposal.

A letter signed by the Chair of the Institutional Animal Care and Use Committee (IACUC) identifying the proposal submitted to NSBRI by title and certifying approval of the proposed animal research protocols and procedures should be included in the appendix of the proposal. The institution's Public Health Service Animal Welfare Assurance Number must be included on the IACUC certification and entered in the IRB/IACUC section of the Proposal Cover Page. IACUC certifications for other research proposals or grants cannot be substituted (even if they employ the same protocols and procedures).

If IACUC certification is pending on the proposal due date, select "pending" from the IRB/IACUC selection menu on the Proposal Cover Page, and include in the appendix of the proposal a letter signed by the IACUC Chair identifying the proposal by title and indicating the status of the IACUC review process at the time of submission. IACUC certification must be received no later than 90 days after the proposal due date. An application lacking the required IACUC certification 90 days after the proposal due date will be considered incomplete and may be returned to the applicant without review.

NSBRI will require current IACUC certification prior to each year's award.

Duration of Proposed Research – Proposals may be submitted for a duration of one to four years of funding, with an assumed start date of February 1, 2004.

Special Ground Facilities – A variety of special ground research capabilities, including centrifuge facilities, bed rest facilities, etc., are available for use by investigators submitting proposals in response to this NRA. Interested investigators are referred to the *Space Life Sciences Ground Facilities Information Package* for instructions on how to incorporate the use of these facilities into a proposal (see http://research.hq.nasa.gov/code_u/nra/current/NRA-03-OBPR-04/index.html/). Investigators must include the cost of using these facilities in their proposal.

Special Travel and Reporting Requirements – Principal Investigators selected in response to this NRA will be expected to attend two research team meetings, each of two days duration, per year at a location to be determined, as well as one general investigator workshop or retreat per year in the Houston area. Budgets should reflect the costs associated with these meetings and should include a statement indicating that this travel is a special requirement. Selected investigators will become part of the NSBRI's research program and will be expected to provide an annual progress report. Progress is reviewed by the NSBRI's Board of Scientific Counselors (BSC) and reported to NSBRI Management. In addition, investigators will be required to provide annual project information for inclusion in NASA's OBPR Program Tasks and Bibliography. The progress report and Task Book information will be collected electronically.

Data Management Plan – Most data collected through NSBRI support are required to be placed in a central Institute data archive. Investigators should plan to deliver their data to the NSBRI archive **as it is collected**, and should include the cost of such data archiving in their submitted proposal. If selected, a data management plan, including a list of the data products and a schedule for their delivery, must be prepared and submitted to the NSBRI. No additional costs should accompany this plan.

V. Review and Selection Process

Investigators should refer to Appendix A, Section V, for a description of the overall NASA/NSBRI review and selection process.

The selection process will follow the sequence:

1. Review for scientific and technical merit
2. Review for programmatic relevance
3. Selection of Team Leaders and Team Leader projects
4. Selection of remaining proposals for funding

Elements of review and selection unique to the NSBRI are as follows:

NSBRI applications will be evaluated for scientific and technical merit and for the likelihood that the research proposed will have a significant impact on achieving the goals stated in this NRA. As discussed in Appendix A, the initial review will be carried out by an appropriate panel of experts who will discuss and provide a written critique of each proposal. The scientific or technical merit evaluation can be found in Appendix A, Section V of this Announcement. NSBRI proposals scoring in the competitive range will receive a second-level review by NSBRI BSC members. The BSC members will determine how well the research proposal fits with the research priorities enumerated in this Appendix and the Teams' Strategic Plans. These evaluations will be used for programmatic assessment and prioritization, including input from EAC members, newly selected Team Leaders (for those proposals being considered for selection after Team Leaders have been chosen), and NSBRI Management.

The programmatic relevance assessment will include an evaluation of how the proposed work will help achieve an appropriate balance of scientific and technical tasks required by the critical research issues outlined in the CPR. Assessment of the cost of a proposed effort includes consideration of the appropriateness of the costs and their relationship to available funds.

A set of selection recommendations will be developed by the EAC based on the merit review scores, team relevance, programmatic relevance, and costs. The most important element in the evaluation process is the merit review, which carries the highest weight in final evaluation and selection. The other factors are approximately equal in weight to each other. **Deficiencies in any one of these factors may prevent selection of a proposal.** Proposed selections will be coordinated between the Bioastronautics Research Division at NASA Headquarters and the NSBRI to ensure programmatic balance and elimination of duplicate efforts. Final selections for funding of NSBRI proposals will be made by the NSBRI Director.

Team Leader Application and Selection

Do not put any reference to your interest in being a Team Lead in your proposal. For information only, the following is provided.

To be considered for NSBRI Team Leadership positions, the following criteria must be met in the separate selection process:

1. An application for a Team Leadership position must be submitted using EPSS;
2. A separate research proposal in response to this appendix of this NRA must be submitted;
3. Proposal must receive a peer review score that places it within the competitive range for scientific/technical merit; and
4. Proposal must be clearly relevant to the NSBRI research team to which applicant is applying.

For applications that meet these four criteria, the NSBRI EAC will carry out the following:

1. Consider the peer review panel comments on the scientific and technical merit of each qualifying proposal;
2. Consider the comments of the BSC concerning team relevancy;
3. Evaluate the merits of Team Leader applicants using the Team Leadership criteria identified in the Call for Candidates Solicitation; and
4. Based on the aforementioned criteria 1-3, make recommendations to the NSBRI Director.

The NSBRI Director will make nominations for Team Leadership positions, which require approval by the NSBRI Board of Directors. Selection of Team Leader projects will be made by the NSBRI Director in coordination with NASA.

Research proposals from unsuccessful Team Leader applicants will be returned with the other proposals for potential selection for funding.

Original signed by _____
Bobby R. Alford, M.D., Chairman of the Board and CEO
NSBRI

**CERTIFICATION REGARDING DEBARMENT, SUSPENSION, AND OTHER
RESPONSIBILITY MATTERS**

PRIMARY COVERED TRANSACTIONS

This certification is required by the regulations implementing Executive Order 12549, Debarment and Suspension, 14 CFR Part 1269.

A. The applicant certifies that it and its principals:

- (a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;
- (b) Have not within a three-year period preceding this application been convicted or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State, or Local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;
- (c) Are not presently indicted for or otherwise criminally or civilly charged by a government entity (Federal, State, or Local) with commission of any of the offenses enumerated in paragraph A.(b) of this certification; and
- (d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State, or Local) terminated for cause or default; and

B. Where the applicant is unable to certify to any of the statements in this certification, he or she shall attach an explanation to this application.

C. Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lowered Tier Covered Transactions (Subgrants or Subcontracts)

- a) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principles is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any federal department of agency.
- b) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

**CERTIFICATION REGARDING
LOBBYING**

As required by S 1352 Title 31 of the U.S. Code for persons entering into a grant or cooperative agreement over \$100,000, the applicant certifies that:

- (a) No Federal appropriated funds have been paid or will be paid by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, in connection with making of any Federal grant, the entering into of any cooperative, and the extension, continuation, renewal, amendment, or modification of any Federal grant or cooperative agreement;
- (b) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting an officer or employee of any agency, Member of Congress, an or an employee of a Member of Congress in connection with this Federal grant or cooperative agreement, the undersigned shall complete Standard Form - LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.
- (c) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subgrants, contracts under grants and cooperative agreements, and subcontracts), and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by S1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

**CERTIFICATION OF COMPLIANCE WITH THE NASA REGULATIONS PURSUANT
TO
NONDISCRIMINATION IN FEDERALLY ASSISTED PROGRAMS**

The (Institution, corporation, firm, or other organization on whose behalf this assurance is signed, hereinafter called "Applicant") hereby agrees that it will comply with Title VI of the Civil Rights Act of 1964 (P.L. 88-352), Title IX of the Education Amendments of 1962 (20 U.S. 1680 et seq.), Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S. 794), and the Age Discrimination Act of 1975 (42 U.S. 16101 et seq.), and all requirements imposed by or pursuant to the Regulation of the National Aeronautics and Space Administration (14 CFR Part 1250) (hereinafter called "NASA") issued pursuant to these laws, to the end that in accordance with these laws and regulations, no person in the United States shall, on the basis of race, color, national origin, sex, handicapped condition, or age be excluded from participating in, be denied the benefits of, or be otherwise subjected to discrimination under any program or activity for which the Applicant receives federal financial assistance from NASA; and hereby give assurance that it will immediately take any measure necessary to effectuate this agreement.

If any real property or structure thereon is provided or improved with the aid of federal financial assistance extended to the Applicant by NASA, this assurance shall obligate the Applicant, or in the case of any transfer of such property, any transferee, for the period during which the real property or structure is used for a purpose for which the federal financial assistance is extended or for another purpose involving the provision of similar services or benefits. If any personal property is so provided, this assurance shall obligate the Applicant for the period during which the federal financial assistance is extended to it by NASA.

This assurance is given in consideration of and for the purpose of obtaining any and all federal grants, loans, contracts, property, discounts, or other federal financial assistance extended after the date hereof to the Applicant by NASA, including installment payments after such date on account of applications for federal financial assistance which were approved before such date. The Applicant recognized and agrees that such federal financial assistance will be extended in reliance on the representations and agreements made in this assurance, and the United States shall have the right to seek judicial enforcement of this assurance. His assurance is binding on the Applicant, its successors, transferees, and assignees, and the person or persons whose signatures appear below are authorized to sign on behalf of the Applicant.

INSTRUCTIONS FOR RESPONDING TO NASA RESEARCH ANNOUNCEMENTS
(MAY 2002)

(a) General.

(1) Proposals received in response to a NASA Research Announcement (NRA) will be used only for evaluation purposes. NASA does not allow a proposal, the contents of which are not available without restriction from another source, or any unique ideas submitted in response to an NRA to be used as the basis of a solicitation or in negotiation with other organizations, nor is a pre-award synopsis published for individual proposals.

(2) A solicited proposal that results in a NASA award becomes part of the record of that transaction and may be available to the public on specific request; however, information or material that NASA and the awardee mutually agree to be of a privileged nature will be held in confidence to the extent permitted by law, including the Freedom of Information Act.

(3) NRAs contain programmatic information and certain requirements which apply only to proposals prepared in response to that particular announcement. These instructions contain the general proposal preparation information which applies to responses to all NRAs.

(4) A contract, grant, cooperative agreement, or other agreement may be used to accomplish an effort funded in response to an NRA. NASA will determine the appropriate award instrument. Contracts resulting from NRAs are subject to the Federal Acquisition Regulation and the NASA FAR Supplement. Any resultant grants or cooperative agreements will be awarded and administered in accordance with the NASA Grant and Cooperative Agreement Handbook (NPG 5800.1).

(5) NASA does not have mandatory forms or formats for responses to NRAs; however, it is requested that proposals conform to the guidelines in these instructions. NASA may accept proposals without discussion; hence, proposals should initially be as complete as possible and be submitted on the proposers' most favorable terms.

(6) To be considered for award, a submission must, at a minimum, present a specific project within the areas delineated by the NRA; contain sufficient technical and cost information to permit a meaningful evaluation; be signed by an official authorized to legally bind the submitting organization; not merely offer to perform standard services or to just provide computer facilities or services; and not significantly duplicate a more specific current or pending NASA solicitation.

(b) NRA-Specific Items. Several proposal submission items appear in the NRA itself: the unique NRA identifier; when to submit proposals; where to send proposals; number of copies required; and sources for more information. Items included in these instructions may be supplemented by the NRA.

(c) The following information is needed to permit consideration in an objective manner. NRAs will generally specify topics for which additional information or greater detail is desirable. Each proposal copy shall contain all submitted material, including a copy of the transmittal letter if it contains substantive information.

(1) Transmittal Letter or Prefatory Material.

- (i) The legal name and address of the organization and specific division or campus identification if part of a larger organization;
- (ii) A brief, scientifically valid project title intelligible to a scientifically literate reader and suitable for use in the public press;
- (iii) Type of organization: e.g., profit, nonprofit, educational, small business, minority, women-owned, etc.;
- (iv) Name and telephone number of the principal investigator and business personnel who may be contacted during evaluation or negotiation;
- (v) Identification of other organizations that are currently evaluating a proposal for the same efforts;
- (vi) Identification of the NRA, by number and title, to which the proposal is responding;
- (vii) Dollar amount requested, desired starting date, and duration of project;
- (viii) Date of submission; and
- (ix) Signature of a responsible official or authorized representative of the organization, or any other person authorized to legally bind the organization (unless the signature appears on the proposal itself).

(2) Restriction on Use and Disclosure of Proposal Information. Information contained in proposals is used for evaluation purposes only. Offerors or quoters should, in order to maximize protection of trade secrets or other information that is confidential or privileged, place the following notice on the title page of the proposal and specify the information subject to the notice by inserting an appropriate identification in the notice. In any event, information contained in proposals will be protected to the extent permitted by law, but NASA assumes no liability for use and disclosure of information not made subject to the notice.

Notice

Restriction on Use and Disclosure of Proposal Information

The information (data) contained in [insert page numbers or other identification] of this proposal constitutes a trade secret and/or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that it will not, without permission of the offeror, be used or disclosed other than for evaluation purposes; provided, however, that in the event a contract (or other agreement) is awarded on the basis of this proposal the Government shall have the right to use and disclose this information (data) to the extent provided in the contract (or other agreement). This restriction does not limit the Government's right to use or disclose this information (data) if obtained from another source without restriction.

(3) Abstract. Include a concise (200-300 word if not otherwise specified in the NRA) abstract describing the objective and the method of approach.

(4) Project Description.

(i) The main body of the proposal shall be a detailed statement of the work to be undertaken and should include objectives and expected significance; relation to the present state of knowledge; and relation to previous work done on the project and to related work in progress elsewhere. The statement should outline the plan of work, including the broad design of experiments to be undertaken and a description of experimental methods and procedures. The project description should address the evaluation factors in these instructions and any specific factors in the NRA. Any substantial collaboration with individuals not referred to in the budget or use of consultants should be described. Subcontracting significant portions of a research project is discouraged.

(ii) When it is expected that the effort will require more than one year, the proposal should cover the complete project to the extent that it can be reasonably anticipated. Principal emphasis should be on the first year of work, and the description should distinguish clearly between the first year's work and work planned for subsequent years.

(5) Management Approach. For large or complex efforts involving interactions among numerous individuals or other organizations, plans for distribution of responsibilities and arrangements for ensuring a coordinated effort should be described.

(6) Personnel. The principal investigator is responsible for supervision of the work and participates in the conduct of the research regardless of whether or not compensated under the award. A short biographical sketch of the principal investigator, a list of principal publications and any exceptional qualifications should be included. Omit social security number and other personal items which do not merit consideration in evaluation of the proposal. Give similar biographical information on other senior professional personnel who will be directly associated with the project. Give the names and titles of any other scientists and technical personnel associated substantially with the project in an advisory capacity. Universities should list the approximate number of students or other assistants, together with information as to their level of academic attainment. Any special industry-university cooperative arrangements should be described.

(7) Facilities and Equipment.

(i) Describe available facilities and major items of equipment especially adapted or suited to the proposed project, and any additional major equipment that will be required. Identify any Government-owned facilities, industrial plant equipment, or special tooling that are proposed for use. Include evidence of its availability and the cognizant Government points of contact.

(ii) Before requesting a major item of capital equipment, the proposer should determine if sharing or loan of equipment already within the organization is a feasible alternative. Where such arrangements cannot be made, the proposal should so state. The need for items that typically can be used for research and non-research purposes should be explained.

(8) Proposed Costs (U.S. Proposals Only).

(i) Proposals should contain cost and technical parts in one volume: do not use separate "confidential" salary pages. As applicable, include separate cost estimates for salaries and wages; fringe benefits; equipment; expendable materials and supplies; services; domestic and foreign travel; ADP expenses; publication or page charges; consultants; subcontracts; other miscellaneous identifiable direct costs; and indirect costs. List salaries and wages in appropriate organizational categories (e.g., principal investigator, other scientific and engineering professionals, graduate

students, research assistants, and technicians and other non-professional personnel). Estimate all staffing data in terms of staff-months or fractions of full-time.

(ii) *Explanatory notes should accompany the cost proposal to provide identification and estimated cost of major capital equipment items to be acquired; purpose and estimated number and lengths of trips planned; basis for indirect cost computation (including date of most recent negotiation and cognizant agency); and clarification of other items in the cost proposal that are not self-evident. List estimated expenses as yearly requirements by major work phases.*

(iii) Allowable costs are governed by FAR Part 31 and the NASA FAR Supplement Part 1831 (and OMB Circulars A-21 for educational institutions and A-122 for nonprofit organizations).

(iv) Use of NASA funds--NASA funding may not be used for foreign research efforts at any level, whether as a collaborator or a subcontract. The direct purchase of supplies and/or services, which do not constitute research, from non-U.S. sources by U.S. award recipients is permitted. Additionally, in accordance with the National Space Transportation Policy, use of a non-U.S. manufactured launch vehicle is permitted only on a no-exchange-of-funds basis.

(9) Security. Proposals should not contain security classified material. If the research requires access to or may generate security classified information, the submitter will be required to comply with Government security regulations.

(10) Current Support. For other current projects being conducted by the principal investigator, provide title of project, sponsoring agency, and ending date.

(11) Special Matters.

(i) Include any required statements of environmental impact of the research, human subject or animal care provisions, conflict of interest, or on such other topics as may be required by the nature of the effort and current statutes, executive orders, or other current Government-wide guidelines.

(ii) Proposers should include a brief description of the organization, its facilities, and previous work experience in the field of the proposal. Identify the cognizant Government audit agency, inspection agency, and administrative contracting officer, when applicable.

(d) Renewal Proposals.

(1) Renewal proposals for existing awards will be considered in the same manner as proposals for new endeavors. A renewal proposal should not repeat all of the information that was in the original proposal. The renewal proposal should refer to its predecessor, update the parts that are no longer current, and indicate what elements of the research are expected to be covered during the period for which support is desired. A description of any significant findings since the most recent progress report should be included. The renewal proposal should treat, in reasonable detail, the plans for the next period, contain a cost estimate, and otherwise adhere to these instructions.

(2) NASA may renew an effort either through amendment of an existing contract or by a new award.

(e) **Length.** Unless otherwise specified in the NRA, effort should be made to keep proposals as brief as possible, concentrating on substantive material. Few proposals need exceed 15-20 pages. Necessary detailed information, such as reprints, should be included as attachments. A complete set of attachments is necessary for each copy of the proposal. As proposals are not returned, avoid use of "one-of-a-kind" attachments.

(f) Joint Proposals.

(1) Where multiple organizations are involved, the proposal may be submitted by only one of them. It should clearly describe the role to be played by the other organizations and indicate the legal and managerial arrangements contemplated. In other instances, simultaneous submission of related proposals from each organization might be appropriate, in which case parallel awards would be made.

(2) Where a project of a cooperative nature with NASA is contemplated, describe the contributions expected from any participating NASA investigator and agency facilities or equipment which may be required. The proposal must be confined only to that which the proposing organization can commit itself. "Joint" proposals which specify the internal arrangements NASA will actually make are not acceptable as a means of establishing an agency commitment.

(g) **Late Proposals.** Proposals or proposal modifications received after the latest date specified for receipt may be considered if a significant reduction in cost to the Government is probable or if there are significant technical advantages, as compared with proposals previously received.

(h) **Withdrawal.** Proposals may be withdrawn by the proposer at any time before award. Offerors are requested to notify NASA if the proposal is funded by another organization or of other changed circumstances which dictate termination of evaluation.

(i) Evaluation Factors.

(1) Unless otherwise specified in the NRA, the principal elements (of approximately equal weight) considered in evaluating a proposal are its relevance to NASA's objectives, intrinsic merit, and cost.

(2) Evaluation of a proposal's relevance to NASA's objectives includes the consideration of the potential contribution of the effort to NASA's mission.

(3) Evaluation of its intrinsic merit includes the consideration of the following factors of equal importance:

(i) Overall scientific or technical merit of the proposal or unique and innovative methods, approaches, or concepts demonstrated by the proposal.

(ii) Offeror's capabilities, related experience, facilities, techniques, or unique combinations of these which are integral factors for achieving the proposal objectives.

(iii) The qualifications, capabilities, and experience of the proposed principal investigator, team leader, or key personnel critical in achieving the proposal objectives.

(iv) Overall standing among similar proposals and/or evaluation against the state-of-the-art.

(4) Evaluation of the cost of a proposed effort may include the realism and reasonableness of the proposed cost and available funds.

(j) Evaluation Techniques. Selection decisions will be made following peer and/or scientific review of the proposals. Several evaluation techniques are regularly used within NASA. In all cases proposals are subject to scientific review by discipline specialists in the area of the proposal. Some proposals are reviewed entirely in-house, others are evaluated by a combination of in-house and selected external reviewers, while yet others are subject to the full external peer review technique (with due regard for conflict-of-interest and protection of proposal information), such as by mail or through assembled panels. The final decisions are made by a NASA selecting official. A proposal which is scientifically and programmatically meritorious, but not selected for award during its initial review, may be included in subsequent reviews unless the proposer requests otherwise.

(k) Selection for Award.

(1) When a proposal is not selected for award, the proposer will be notified. NASA will explain generally why the proposal was not selected. Proposers desiring additional information may contact the selecting official who will arrange a debriefing.

(2) When a proposal is selected for award, negotiation and award will be handled by the procurement office in the funding installation. The proposal is used as the basis for negotiation. The contracting officer may request certain business data and may forward a model award instrument and other information pertinent to negotiation.

(l) Additional Guidelines Applicable to Foreign Proposals and Proposals Including Foreign Participation.

(1) NASA welcomes proposals from outside the U.S. However, foreign entities are generally not eligible for funding from NASA. Therefore, unless otherwise noted in the NRA, proposals from foreign entities should not include a cost plan unless the proposal involves collaboration with a U.S. institution, in which case a cost plan for only the participation of the U.S. entity must be included. Proposals from foreign entities and proposals from U.S. entities that include foreign participation must be endorsed by the respective government agency or funding/sponsoring institution in the country from which the foreign entity is proposing. Such endorsement should indicate that the proposal merits careful consideration by NASA, and if the proposal is selected, sufficient funds will be made available to undertake the activity as proposed.

(2) All foreign proposals must be typewritten in English and comply with all other submission requirements stated in the NRA. All foreign proposals will undergo the same evaluation and selection process as those originating in the U.S. All proposals must be received before the established closing date. Those received after the closing date will be treated in accordance with paragraph (g) of this provision. Sponsoring foreign government agencies or funding institutions may, in exceptional situations, forward a proposal without endorsement if

endorsement is not possible before the announced closing date. In such cases, the NASA sponsoring office should be advised when a decision on endorsement can be expected.

(3) Successful and unsuccessful foreign entities will be contacted directly by the NASA sponsoring office. Copies of these letters will be sent to the foreign sponsor. Should a foreign proposal or a U.S. proposal with foreign participation be selected, NASA's Office of External Relations will arrange with the foreign sponsor for the proposed participation on a no-exchange-of-funds basis, in which NASA and the non-U.S. sponsoring agency or funding institution will each bear the cost of discharging their respective responsibilities.

(4) Depending on the nature and extent of the proposed cooperation, these arrangements may entail:

- (i) An exchange of letters between NASA and the foreign sponsor; or
- (ii) A formal Agency-to-Agency Memorandum of Understanding (MOU).

(m) **Cancellation of NRA.** NASA reserves the right to make no awards under this NRA and to cancel this NRA. NASA assumes no liability for canceling the NRA or for anyone's failure to receive actual notice of cancellation.

**Proposal Submission Frequently Asked Questions (FAQs)
and Sample Forms**
(Independent Investigator Research Projects Only)

The information provided here is in response to questions from investigators such as yourself. Additional information regarding submission procedures and requirements can be found in the research announcement to which you are responding, and at the NASA online proposal site:

<http://proposals.hq.nasa.gov/proposal.cfm>

1. What forms should I use when submitting a proposal?

Currently, the NASA proposal site does not support the uploading of information or forms other than the information gathered while completing the online cover page. Please complete the online cover page early in the process (you can always return and edit the cover page at any time up to the due date). After completing the cover page, any additional information you are required to provide or wish to provide can be submitted in hardcopy in any format you choose.

Please find included in this document several sample forms that you may use when providing additional information. A standard checklist of materials to include is also provided. Information outside of the online proposal cover page can be provided in any format you choose, as long as it adheres to the NRA requirements. Please reference the NRA for information on all material required when submitting your proposal. Please be aware that we ask for copies of the completed proposal package, not just the project description, and must **receive** the copies by the proposal due date. The additional information requested in the NRA does not count towards the 20 page limit of your project description.

2. Where does my authorizing official sign?

You must include your authorizing official as a team member. When you complete and print the proposal cover page, you will see signature blocks both for yourself and your authorizing official. You are required to submit one original signed (by both you and your authorizing official) cover page with your proposal hardcopies.

To be added as a team member to your proposal, the individual must be registered with the SYS-EYFUS system. If you try and add a team member and they are not found in the database, you must contact and have that individual register as a new SYS-EYFUS user. You will then be able to add them as a team member.

3. Who should I contact if I receive errors or have additional problems while using the NASA proposal site?

For technical support, please e-mail proposals@hq.nasa.gov or call 202-479-9376 (Monday to Friday 8 a.m.-6 p.m. EST/EDT).

CHECKLIST FOR PROPOSERS

(Independent Investigator Research Projects Only)

- Proposal Cover Page (completed online)
- Response to previous reviews (if applicable)
- Project Description
- Biographical Sketches
- Other Support
- Facilities and Equipment Description
- Summary Budget Form/Budget Justification
- Detailed 12-Month Budget (for each year of support)
- IRB or ACUC letter/form (if applicable)
- Letters of Collaboration/Support (if applicable)
- Appendices, if any

Form B

(Independent Investigator Research Projects Only)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
------	----------------

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).

INSTITUTION(S) AND LOCATION	DEGREE(S) (if applicable)	YEAR(S)	FIELD(S) OF STUDY

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years, and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

(Independent Investigator Research Projects Only)

BUDGET FOR ENTIRE PROJECT PERIOD

DIRECT COSTS ONLY

<i>BUDGET CATEGORY TOTALS</i>		<i>1st BUDGET PERIOD</i>	<i>ADDITIONAL YEARS OF SUPPORT REQUESTED</i>		
			<i>2nd</i>	<i>3rd</i>	<i>4th</i>
PERSONNEL (Salary and Fringe Benefits) (Applicant organization only)					
SUBCONTRACTS					
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES					
TRAVEL	DOMESTIC				
	NON-DOMESTIC				
OTHER EXPENSES					
TOTAL DIRECT COSTS FOR EACH PERIOD					
TOTAL INDIRECT COSTS FOR EACH PERIOD					
TOTAL DIRECT + INDIRECT COSTS FOR EACH PERIOD					
TOTAL DIRECT + INDIRECT COSTS FOR ENTIRE PROJECT					

JUSTIFICATION FOR UNUSUAL EXPENSES :

Form D

(Independent Investigator Research Projects Only)

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD		FROM	THROUGH		
DIRECT COSTS ONLY					
Duplicate this form for each year of grant support requested		FUNDING AMOUNT REQUESTED			
PERSONNEL (Applicant Organization Only)					
NAME	ROLE IN PROJECT	EFFORT ON PROJECT	SALARY	FRINGE BENEFITS	TOTALS
	Principal Investigator				
SUBTOTALS →					
SUBCONTRACTS					
CONSULTANT COSTS					
EQUIPMENT (Itemize; use additional sheet if needed)					
SUPPLIES (Itemize by category; use additional sheet if needed)					
TRAVEL	DOMESTIC				
	NON-DOMESTIC				
OTHER EXPENSES (Itemize by category; use additional sheet if needed)					
TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD					
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD					
TOTAL COST FOR FIRST 12-MONTH BUDGET PERIOD					

OTHER SUPPORT

(Independent Investigator Research Projects Only)

Please provide information regarding specific sources of other support for the principal investigator and each co-investigator (not consultants). This information should be provided separately for each individual in the format shown below. List all active support for an individual before listing pending support. Include the investigator's name at the top of each page and number pages consecutively.

NAME OF INDIVIDUAL		
ACTIVE/PENDING		
Project Number (Principal Investigator)	Dates of Approved/ Proposed Project	Percent Effort
Source	Annual Direct Costs	
Title of Project (or Subproject)		
One-sentence description of project goals. (The major goals of this project are...)		
Brief description of potential scientific or commitment overlap with respect to this individual between this application and projects described above (summarized for each individual).		

CRITICAL PATH ROADMAP (CPR) FORM

(Independent Investigator Research Projects and NSBRI Team Research Projects)

Hypotheses	Risk Number (from Critical Path Roadmap)	Critical Question Number (from Critical Path Roadmap)	Critical Question (from Critical Path Roadmap)	Specific Aim

Appendix H



April 15, 2003
NSBRI CFC-03-01

National Space Biomedical Research Institute
One Baylor Plaza, NA-425
Houston, TX 77030

NSBRI Call for Candidates
Soliciting Applications for Team
Leadership

A Research Announcement for the
National Space Biomedical Research Institute

Applications Due: July 15, 2003

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NSBRI Call for Candidates Soliciting Applications for Team Leadership

Summary and Supplemental Information

The NSBRI is a private, non-profit organization competitively selected by NASA. The Institute uses an integrated research team approach to advance biomedical research with the goal of ensuring safe and productive long-term human exploration of space.

The NSBRI is responsible for the development of countermeasures against the deleterious effects of long-duration space flight and applied space biomedical research directed toward this specific goal. Its mission is to lead a national effort in integrated, critical path space biomedical research that supports NASA's Bioastronautics Strategy by focusing on the enabling of long-term human presence in, development of, and exploration of space. This is accomplished by:

- designing and testing effective countermeasures to address the biological and environmental impediments to long-term human space flight;
- defining the molecular, cellular, organ-level, and integrated responses and mechanistic relationships that ultimately determine these impediments, where such activity is essential for the development of novel countermeasures;
- establishing biomedical support technologies to maximize human performance in space, reducing biomedical hazards to an acceptable level, and delivering quality medical care;
- transferring and disseminating the biomedical advances in knowledge and technology to the general benefit of mankind; and
- ensuring open involvement of a diverse scientific community, industry, and the public at large in the Institute's activities and fostering a robust partnership with NASA, particularly through NASA's Lyndon B. Johnson Space Center.

Each of the NSBRI research teams consists of a set of coordinated and complementary projects focused on a common theme. Team management and coordination is the responsibility of the **Team Leader**. A single Team Leader, assisted by an Associate Team Leader, heads each research team. This Call for Candidates solicitation is for applications for Team Leadership positions.

The following items apply only to this Call for Candidates (CFC):

Solicitation Identifier:	NSBRI CFC-03-01
Submission Format:	Electronic applications utilizing the NSBRI's Electronic Proposal Submission System
Notices of Intent Due:	N/A
Applications Due:	July 15, 2003

Information about the NSBRI and its existing research teams is available from:

Jeffrey P. Sutton, M.D., Ph.D.
Director, National Space Biomedical Research Institute
One Baylor Plaza, NA-425
Houston, TX 77030-3498
Telephone: 713-798-7412
Fax: 713-798-7413
Email: director@www.nsbri.org

Original signed by

Bobby R. Alford, M.D.
Chairman of the Board and CEO
National Space Biomedical Research Institute

National Space Biomedical Research Institute Call for Candidates

NOTE: Team Leadership positions for Institute research teams will be competed in parallel with NRA 03-OBPR-04. All applicants for this CFC must submit a proposal as a Principal Investigator to Appendix C of NRA 03-OBPR-04. The Institute's Policy on Team Leadership is contained in Appendix B of this document.

I. Introduction

Team Leaders play a pivotal role in guiding the Institute's research program and in the ultimate success of the Institute. Their expertise and "hands-on" approach to research management add value across projects and across teams. The Team Leader is guided by the Critical Path Roadmap, which is the cornerstone for developing the team's integrated strategic research plan, the key to accomplishing the Institute's mission. The Team Leader's stature and reputation as a strong scientist encourages other scientists to apply to become team members. The Team Leader's communication skills and insight enable the appropriate synergistic discussions among the various research projects, with the objective of assuring a team research program that has higher value than the sum of the values of its separate projects. **The NSBRI invites applications to lead an existing team in one of 10 research areas:**

1. *Bone Loss* – Addressing bone loss and weakening during space flight and the inherent fracture risks.
2. *Cardiovascular Alterations* – Addressing the in-flight occurrence of cardiac dysrhythmias and post-flight impairment of the cardiovascular response to orthostatic and exercise stress.
3. *Human Performance Factors, Sleep and Chronobiology* – Investigating maintenance of high cognitive performance and vigilance despite environmental stress and sleep disturbances.
4. *Immunology, Infection and Hematology* – Addressing immune system impairment and altered susceptibility to infection, increased allergic responsiveness, decreased blood volume, and post-flight anemia.
5. *Muscle Alterations and Atrophy* – Focusing on the loss of skeletal muscle mass, strength, and endurance that accompanies space flight.
6. *Neurobehavioral and Psychosocial Factors* – Investigating methods and tools that can be utilized to enable crews to cope with stress, isolation, and compatibility.
7. *Neurovestibular Adaptation* – Addressing the problems of space motion sickness and disorientation during flight and the post-flight problems of balance and gaze disorders.
8. *Nutrition, Physical Fitness, and Rehabilitation* – Developing methods to maintain health and fitness before, during, and after space flights.
9. *Smart Medical Systems* – Developing new methods of non-invasive medical monitoring, diagnosis, and therapy for use on space missions.

10. *Technology Development* – Developing instrumentation and other technological products that will enhance the research of the other teams and benefit people on Earth.

Applicants are requested to carefully review the NSBRI Policy on Team Leadership (Appendix B) prior to submitting an application.

II. Application Requirements

This is an open solicitation. The NSBRI is soliciting applications for team leadership positions on a competitive basis. **Applicants must prepare and submit in response to NRA 03-OBPR-04, as Principal Investigator, a proposal that achieves a merit score in the competitive range as a prerequisite for being considered for a Team Leadership position.** Current Team Leaders may reapply for the next term. All applications for Team Leadership positions will be considered new applications. A separate application process from NRA 03-OBPR-04, outlined in this Call for Candidates, will be used to select Team Leadership. **Do not make any reference to your interest in Team Leadership in your NSBRI research proposal application to NRA 03-OBPR-04.**

III. Application Procedures

Applications for Team Leadership positions must be submitted through NSBRI's Internet-based Electronic Proposal Submission System (EPSS).

A Notice of Intent to apply is not necessary for Team Leadership applications. To prepare the application for Team Leadership, go to the Web site <http://myportal.nsbri.org> and register to obtain a personal account on the system if you do not already have one. After entering contact information, applicants will receive a username and password for entry into EPSS. After this, the above Web address will serve as the entry point for application development and modification. All information entered will remain private until electronic submission is completed.

An application overview screen will guide applicants through the process of completing the required information. There are four main sections applicants will be required to complete prior to electronic submission. The four application questions are:

1. What is your motivation to apply for and commitment to serve in an NSBRI Team Leadership position?
2. What is your vision for the team for the next 5 years?
3. What previous scientific experience qualifies you for a Team Leadership position?
4. What previous management experience qualifies you for a Team Leadership position?

Each section has a character limit of 2,500 characters.

All investigators can allow an administrative support person to act on their behalf, to assist in the entry of application information; however, electronic submission can only be performed by the applicant.

Electronic applications must be submitted before 5:00 p.m. EST, Tuesday, July 15, 2003. After submission using EPSS, the applicant **must** sign and mail the printed application cover

page that is generated by the system to the following address within **one week** of the submission deadline:

NSBRI, *Attn: NSBRI CFC-03-01*
One Baylor Plaza, NA-425
Houston, TX 77030-3498
713-798-7412

Please direct any questions concerning this application procedure to the NSBRI by calling 713-798-7412, by faxing your questions to 713-798-7413, or by sending your inquiry to contact_us@www.nsbri.org. The technical requirements to operate EPSS are Internet Explorer 4.0+ or Netscape 4.03+ for Windows, Macintosh, or Unix. EPSS is best viewed using Internet Explorer 6.0.

IV. Evaluation

Only applications electronically submitted by EPSS will be evaluated. The four sections of the application will be reviewed using the criteria set forth in the NSBRI Policy on Team Leadership (Appendix B). Each section will carry equal weight in the evaluation process.

V. Selection Process

To be considered for an NSBRI Team Leadership position, the following criteria must be met:

1. An application for a Team Leadership position must be submitted using EPSS;
2. A separate research proposal in response to Appendix C of NRA 03-OBPR-04 must be submitted;
3. The research proposal must receive a peer review score that places it within the competitive range for scientific/technical merit; and
4. The research proposal must be clearly relevant to the NSBRI research team to which the applicant is applying.

For applications that meet these four criteria, the NSBRI External Advisory Council will carry out the following:

1. Consider the peer review panel comments on the scientific and technical merit of each qualifying proposal;
2. Consider the comments of the Board of Scientific Counselors concerning team relevancy;
3. Evaluate the merits of Team Leadership applicants using the Team Leadership criteria identified in this Call for Candidates Solicitation (Appendix B); and
4. Based on the aforementioned criteria 1-3, make recommendations to the NSBRI Director.

The NSBRI Director will then make nominations for Team Leadership positions, which require approval by the NSBRI Board of Directors. Selection of Team Leader projects will be made by the NSBRI Director in coordination with NASA.

Research proposals from unsuccessful Team Leadership applicants will be considered with the other proposals for potential selection for funding.

**NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
POLICY ON TEAM LEADERSHIP**

I. Overview

Each Institute team is led by a single Team Leader who is assisted by an Associate Team Leader. Team Leaders play a pivotal role in guiding the Institute's research program and the ultimate success of the Institute. Their expertise and "hands-on" approach to research management add value across projects and across teams. The Team Leader is guided by the Critical Path Roadmap (CPR), which is the cornerstone for developing the team's integrated strategic research plan, the key to accomplishing the Institute's mission. The Team Leader's stature and reputation as a strong scientist encourages other scientists to apply to become team members. The Team Leader's communication skills and insight enable the appropriate synergistic discussions among the various research projects, with the objective of assuring a team research program that has higher value than the sum of the values of its separate projects.

II. Duties and Responsibilities

Team Leaders are responsible for:

- Preparing and periodically updating the team research strategic plan. This plan should be consistent with the Institute mission, the CPR, and available resources.
- Reporting progress to the Institute's External Advisory Council (EAC), Board of Scientific Counselors (BSC), and NSBRI management.
- Preparing and presenting the initial recommendation to the EAC of new research projects for inclusion in the team's program.
- Representing the team and disseminating knowledge about team activities and progress to NASA; specifically coordinating with NASA-JSC scientists and physicians, the scientific community, and the general public.
- Pursuing involvement with NASA operational activities.
- Maintaining appropriate communication links among the team investigators and to other team leaders.
- Developing, with team investigators, individual project plans that ensure scientific and operational synergy and lead to productive countermeasure development.
- Nurturing opportunities and seeking funding support to collaborate with, and cross-fertilize, research within and between NSBRI teams and with Johnson Space Center, other NASA Centers, and other agencies.
- Acting as the senior NSBRI discipline representative for ongoing development of the CPR.

Associate Team Leaders assist Team Leaders in carrying out the above activities.

III. Qualifications

Team Leaders are NSBRI-funded principal investigators who possess the following qualifications:

- Achieved intermediate or senior rank at a research or educational institution.
- Demonstrated record of securing independent competitive research funding for the last five years, at least.
- Recognized within the biomedical community as an outstanding research contributor to at least one field of study; prior involvement with a NASA flight investigation would be beneficial.
- Manifest broad scientific understanding across the team's research area.
- Demonstrated leadership and program/group management skills, as evidenced by experiences such as a section head, department chair, dean, research center director, or principal investigator on a program project.
- Exhibit good communication, public speaking, and organizational skills.
- Show a willingness and availability to spend the necessary time and energy to fulfill the role of Team Leader.

Associate Team Leaders are principal or co-investigators on NSBRI-funded projects who possess at least the first four of the above qualifications required for a Team Leader. Generally, Team Leaders and Associate Team Leaders are not from the same institution.

IV. Term of Service

Team Leaders are appointed by the Director for a term that is identical with the term of their NSBRI-funded research project (generally four years), subject to satisfactory performance as determined at their annual performance review. The Team Leader appoints an Associate Team Leader for a term that does not exceed the Team Leader's term of service. The Team Leader's term is competitively renewable.

V. Funding and Authority

Team Leaders and Associate Team Leaders are provided with discretionary funds to enable them to carry out their duties and responsibilities. Wide latitude is provided concerning the expenditure of these funds within the guidelines of the involved institutions. Such funds may be used for support personnel, team meetings, special travel, and other expenses generally associated with team communication and operations. However, these funds may not be used to support research.

Team Leaders are ultimately responsible for carrying out the duties and responsibilities listed in Section II. They are expected to work cooperatively with their Associate Team Leader in all matters and should develop a clear understanding of the distribution of their shared responsibilities. Team Leaders report to the Director.

VI. Selection

In the year before a Team Leader's term of service ends, a special "Call for Candidates" Announcement requesting applications for the Team Leader's position will be released in coordination with the annual Institute Research Announcement. Following the

evaluation of the research application by a peer committee, Institute Senior Management (Director and Associate Director) will evaluate, with EAC input, the merits of the applicants for Team Leader. The Director will recommend a selection to the Chairman of the Board who will seek confirmation of the selection from the Board of Directors.

Associate Team Leaders are nominated and selected by the Team Leader, with the advice and consent of the Director in consultation with the Associate Director.

In selection of Team Leaders and Associate Team Leaders, attempts will be made to balance the scientific and managerial expertise of candidates and to develop diversity within the Institute's research leadership.

VII. Training and Support

To assist Team Leaders in performing their duties, the NSBRI provides electronic reporting and managerial tools, along with training as needed. Forums are held at least three times a year for Team Leaders to meet as a group with the Director and Associate Director.

VIII. Performance Evaluation

Once a Team Leader is selected, five groups evaluate the performance and effectiveness of Team Leaders: the EAC, BSC, team principal investigators, NSBRI Senior Management, and NASA. Each group focuses on different aspects of a Team Leader's performance:

- Annually, the BSC will review each team's annual report of productivity and progress in carrying out the team strategy, including evidence that the research projects are functioning synergistically within the research team and evidence that the team is collaborating effectively with other teams and with NASA life scientists.
- Semi-annually, the EAC will review the effectiveness of the Team Leader in communicating the team vision and successes, and in discussing and handling team issues and problems.
- Annually, team principal investigators will evaluate the leadership, communication, and other relevant skills of their Team Leader.
- Annually, Institute Senior Management will evaluate the Team Leader's overall effectiveness and responsiveness.
- At least every four years, and more frequently if necessary, the four research area representatives on the EAC and BSC (two each) will review the team strategic plan and furnish a written critique of the strengths and weaknesses of the plan along with a rating of the overall team strategy embedded in the plan.
- Every five years, just prior to conducting an Institute-wide review, an *ad hoc* review team, appointed by NASA, will evaluate all aspects of the team's performance, including the Team Leader's performance.

Institute Senior Management will produce an annual overall rating of each Team Leader's performance based on the available inputs.

An unsatisfactory Team Leader rating will normally result in a specific warning to the

Team Leader and include a recommended action plan to correct the identified deficiencies in performance. Two unsatisfactory Team Leader ratings in successive years will result in removal of the Team Leader and appointment of an acting Team Leader to serve out the remainder of the Team Leader's term. The Institute supports the need for leadership continuity but only if the evaluative process supports an annual reappointment. Team Leaders are ultimately judged by their team's ability to successfully develop and deliver, in whole or part, countermeasures in areas of high impact for NASA, for the purpose of decreasing the biomedical or human performance risks associated with long-duration human space flight.

Associate Team Leaders are evaluated annually by Team Leaders for their contribution to team goals, achievements, function, productivity, and representation. Unsatisfactory performance may lead to removal of Associate Team Leaders, but such action requires the concurrence of the Director in consultation with the Associate Director.

IX. Conflict of Interest

Team and Associate Team Leaders must adhere to the highest ethical standards as they carry out their leadership duties. They must not make decisions based on institutional affiliation or personal bias. They must conduct all leadership duties with integrity, fairness, and objectivity to ensure the scientific credibility of the Institute.

To avoid a conflict of interest during a selection in which a Team Leader has a competing application, Institute Senior Management selects the Team Leader and project before any other projects are selected. Then the Team Leader develops and presents a selection recommendation concerning the other competing projects to the EAC. The EAC recommends the final selection to Institute Senior Management, taking into account the science merit rating and programmatic relevance rating furnished by the BSC in addition to the Team Leader recommendation. Institute Senior Management makes the final selection decisions following coordination with NASA. If the Team Leader does not have a competing application during a selection cycle, the process is similar but the Team Leader will have input in developing the selection recommendations to the EAC.

Appendix I

April 30, 2003

Proceedings of the Resuscitation and Critical Care Workshop are enclosed. The proceedings include a new educational process for the Crew Medical Officer, the Crew Surgeon, the physician on console in the Mission Control Center (MCC), and the Biomedical Engineer in the MCC. The basics of the curriculum for the Medical Care of the Astronauts in Space (MCAS) training program are covered along with the general principles on which the entire education process was developed. Protocols for the medical management of astronauts in space were revised and recommendations for changes in medications and medical equipment were made. Ethics of medical care in space were addressed.

This report covers the result of more than two years of meetings of the working group. The working group participants included individuals from many elements of the medical community including pre hospital personnel, International Space Station Partners medical experts, flight surgeons, surgeons, emergency physicians, and critical care physicians. Input into the working group deliberations was very broad based.

We are pleased to report to you that this effort is just one of several that emphasize the commitment of the NSBRI and NASA to the development of Space Medicine as a medical discipline and to the continued improvement of medical care in space. As a result of the recommendations of the Critical Care Working Group, a "Pharmacology Summit" working group was formed to address the requirements for medications flown as a part of the medical capability for space flight. Currently, another working group is addressing the training needs for physicians assigned to fly on the ISS and exploration class missions.

The educational curriculum for MCAS has been reviewed and approved by the flight surgeons and the Astronaut Office. Implementation of the MCAS is in progress.

The assistance of those who participated in the Resuscitation and Critical Care Workshop is most appreciated. Additional working group reports will be made available as the work is completed.

Jeffrey Sutton, M.D., Ph.D.
Director,
National Space Biomedical Research Institute

Norman McSwain, M.D., FACS
Chairman, Resuscitation and Critical
Care Working Group

Resuscitation and Critical Care in Space

Executive Summary

CMO RESUSCITATION, CRITICAL CARE & EDUCATION Working group

A working group of physicians, Emergency Medical Technicians-Paramedic (EMT-P) (see attached participant list) to:
discuss the needs of astronauts in orbit on the International Space Station and the Shuttle
and
how the crew medical officers (CMO) could best be prepared to meet this need..

The objectives as amended were

- 1) address the current management structure of the initial critical care and resuscitation of the CMO,
- 2) the management of the most common conditions in space,
- 3) develop protocols for the management of these conditions
- 4) develop changes as necessary in the current medications & supplies flown to carry out these protocols
- 5) development of a training program to provide them with the appropriate education and skills to provide this care.

Methods and process

The working group looked at the current education process, the commonly treated conditions on orbit, including the shuttle-Mir program, the space lab program, the current international space station program and the shuttle missions. The current educational programs for the CMO do not provide consistency in training and the skills and knowledge to address the potential problems on orbit.

Using the list of common known and or expected conditions or orbit. extensive review of the available information on the physiology and micro-gravity, protocols were developed for the overall assessment of patients and then an educational process to address these needs was designed.

Critical in the design of the programs was the recognition that the Soyuz is not an ambulance. The initial plans for the space station included a rapid return vehicle that would be always present with the capable of providing emergency return in a medical or other emergent situation. The emergency return vehicle planning was discontinued, leaving only the Soyuz as the only vehicle available for return.

Soyuz provides very cramped quarters for return, significant $>g=$ forces on landing, and landing zone at an extended distance from definitive medical care. Therefore, the medical conditions need to be managed on the international space station with such circumstances in mind.

Findings

! Training standards

- " Lack of consistency in the education of the CMO
- " Lack educational objectives for the training of the CMO
- " Level of knowledge of the CMO is not consistent with the job requirements
- " Lack distinction between the >need to know= vs the >may need to know= skills and knowledge
- " Lack outcome educational standards have been developed
- " Lack of a plan to teach outcome educational standards
- " Lack of sustainment process for the maintenance of clinical skills and knowledge of the CMO=s
- " Lack of registry of the medical knowledge and skills of the individual CMO=s
- " Lack of sustainment process for the maintenance of clinical skills and knowledge of the Flight surgeons
- " Lack of registry of the knowledge and skills of the individual flight surgeons

! Training process

- " Length of time to provide adequate medical training is not sufficient when compared to other medical care providers with similar responsibilities
- " Lack of testing to assure skill and knowledge maintenance exists prior to flight
- " Lack of adequate time available for skill and knowledge recurrency training prior to flight
- " Lack of defined training officer with responsibility to over see the educational and sustainment process
- " Lack of registry of education and recurrency of the CMO
- " Lack of registry of recurrency of skills and knowledge of the Flight surgeons

! Medication and supplies

- " Better equipment and supplies are currently available to meet the needs of the minimally trained individuals providing care on shuttle and ISS
- " Some of the medications and supplies currently flown are not the most current for the potential conditions that may be encountered
- " The quantity of the medications available are not sufficient to manage the medical conditions that may be encountered and the probable time required to provide the sustained management and to return to out of low earth orbit and access definitive medical care.

! Personnel

- " Lack of training officer with strong oversight authority and time to carry out this mission
- " Lack of associate training officer who has proper background in adult education and curriculum design

! Medical conditions

- " De-orbit is not immediately available and may require 33 hours or so to access medical care therefore such conditions require contingency plans

- " Medical evacuation is the most limiting factor since, for example, a ballistic reentry of 8-10 g=s may exacerbate a medical condition
 - Probabilistic Risk Assessment (PRA) on Soyuz as in Assured Crew Return Vehicle(ACRV), Railsback et al, April 1997
- " Certain medical conditions such as fractures that require immobilization, shock or other condition that will require increased FiO², or need IV fluid administration will not be able to be loaded in to the Soyuz

Recommendations

The recommendations by the working group were:

1) more definitive and prolonged care to be available to provide medical care for more than 3 days in orbit while complicating the need to deorbit.

(This process was recommended 12 years ago in 1991) " ..we have now determined that we=d like to maintain our patient thee for up to 3 days and then, if need be, transport. It gives us a bit of added window..." (*Discussion of the ACRV in the Space Station and Hyperbaric Medicine ad hoc committee meeting September 26-27 1991 page 17*)

2) Provide better training for the CMO to be compatible with providers in other medical professions who are expected to carry out the same or similar skills (Example EMT with ALS skills

3) change in the education process of the crew medical officers to provide such care when the demand arises,

3) Update the currently used medications and supplies to address the above stated needs, and to bring the medications to state of the art in 2002

4) Change in the protocols to provide the above needed patient care

*** Details of the protocol changes are attached.**

*** Details of the recommended medications are attached**

*** Details of the new training program follow**

*Norman McSwain, Jr MD, FACS
Visiting Clinical Professor
Johnson Space Center, NASA*

Medical Care for Astronauts in Space (MCAS) Training program

Objectives of the training program should be:

- 1) to provide efficiency in education to furnish the best skilled individuals in the shortest length of educational time,
- 2) provide consistency in education so that ALL of the students would all have a similar knowledge base,
- 3) assure quality of knowledge base for use of appropriate skills
- 4) assure quality of knowledge for patient assessment.
- 5) teach the skills and knowledge that are them most valuable and most needed to the level of tested quality performance
- 6) teach minimal used skills taught only on an as needed basis >just in time= educational availability on orbit
- 7) frequent testing before orbit to assure that the needed skills are retained and available on orbit..

It is well known that significant skill deterioration occurs with perhaps in less than 25% of the learned levels not present 6-8 months after the initial training if unused.

The current training time allotted for the CMO is inadequate to meet the potential demands of the prolonged missions of greater than 7 days and certainly inadequate when only the Soyuz is available for return. The current training and the allotted time is less than that of the average fireman or police officer who merely responds to an emergency, but provides only rescue first responder type care. It is significantly less than that of the required by federal and state statues for EMT-Basic and the EMT-Paramedic. These individuals are trained to the standards set forth in the National Standard Curriculum that was developed under contract to the U.S. Department of Transportation. This training program includes most of the basic skills required of the crew medical officer. However there are additional skills that the CMO will need that is not included in the National Standard Curriculum and must be added. Such skills require additional training time that is not included in the current educational process.

Some of these skills learned by an EMT, Basic, Intermediate and Paramedic are related to their life on the street just as some of the unique things for the CMO related to their life in space. The bottom line from the prospective of training time is that those life in space components are offset by the life on the street components so that the educational process ant the required hours of training (which have been proven to be effective) for an EMT-Basic should be similar to the educational time provided for the CMO. With the additional invasive skills, the educational process for the CMO will probably be a little bit greater than that of an EMT-Basic, but more like that of an EMT-Intermediate. Approximately 180-200 hours of education will be required to achieve the level of skill and knowledge that the must have.

The educational format will be an adult learning format because the astronaut is a skills driven learner using visual, touch and hearing indicators as their learning rather than reading and conceptual

approaches or the so called kinesthetic learner. Therefore, all of the material developed will be interactive scenario based teach skills first with an indepth learning of the knowledge along with the skills and it will be objective based.

Some portions of the astronauts training program are extremely busy. Others are not as busy. During the 18 months of astronaut candidate school there is some time available, but not a lot. During the mission specific training period there is even less time available but not currently use effectively. The time between completion of the astronaut candidate school and the beginning of the mission specific training however is less constrained and would be the proper place to add the necessary additional education hours. In addition, many skills do not have to be learned indepth as the frequency of use is minimal and they can be learned when needed. A training process that addresses those little used skills that can be trained by audiovisual means can be established in a just in time manner to be utilized in space.

Therefore, the proposed training is divided into four phases:

6. **Basic science phase** done during the astronaut candidate school which would include 40 hours of education divided into 10 segments of 4 hours each. This can be done simultaneously or spread throughout the time of the school. It will address anatomy, physiology, assessment and basic assessment skills. It will also teach the astronaut candidate that basic knowledge that all should know and not necessarily the CMO.
7. **CMO specific training** Approximately 80 hours in length, scenario based. The education is divided as follows:
 - Ten sessions of 4 hour each utilizing the specifically developed objectives (40 hours);
 - Eight hours of skills and assessment with each student practicing on each other.
 - Eight hours in the skills lab and virtual reality (simulator lab),
 - Eight hours in the operating room for endotracheal skills, and assessment of blood, muscle, and sick patients
 - two 8 hour periods in the emergency department,
 - * Four hours in the trauma area,
 - * Four hours in the emergency medicine area.
8. **Mission Specific Education** done in a simulator environment with the training officer, flight surgeon assigned to the mission, and the CMO in separate areas so that the flight surgeon and the CMO gets use to communicating without visual contact. And testing and retraining for skill loss. The length is 33 contact education broken down as follows
 - * 20 hours, one-on-one with crew surgeon (Flight Surgeon assigned to this specific mission). It will include life saving skills and mission specific skills followed by testing and recurrency training as necessary to achieve proficiency.
 - * 13 hours of simulator training. Crew surgeon and CMO interact at a distance with the training officer or assistant training officer observing and critiquing. This is Scenario based

9. **On Orbit training**, just in time training or training which comes through in a CD or DVD format that the astronaut and CMO can review and/or learn just prior to the skill being used.

There are some skills that are life-saving, needs to be immediately recallable, so called reflex skills. Efficiency must be maintained at all times by retraining and testing. Examples of these are CPR and airway management. Other skills which are critical, but do not necessary require reflex are physical examination skills maintained by frequent use, retraining and testing only as necessary. Mission critical and science critical skills such as life and limb salvage, can be refreshed or learned just in time in orbit if necessary. These would be defined as use is likely greater than 2%, knowledge has to be very proficient. Such skills would be IV access and medication. Other minimal use skills which would be all just in time training only introduced on the ground and learned from a video tape if needed would be such things as fracture immobilization and repair of lacerations.

There must be a training officer identified by the Space Medicine Directorate. The individual in this position must spend approximately 80% of his time accomplishing training officer duties. These would include oversee training of the CMO, the flight surgeons, the BMEs and other medical personnel. The medical training officer would also be responsible for assuring that the flight surgeon skills were up-to-date, that they met the necessary continuing education to maintain their skills, and that our records are kept on all medical personnel. To accomplish all of the above duties an assistant training officer would be required. Job descriptions of both are attached.

In summary, basic science information is provided to all astronauts in training in the astronaut candidate area. They will do 40 hours in length in the period between astronaut training and mission specific training. 80 hours of education, including all 4 components of life-saving mission salvage, minimal use, and non-critical frequent use skills will be taught. During the 3rd phase it would be one-on-one refresher training with the flight surgeon and use of simulations. The 4th phase would be just in time training.

Benefits to NASA would be:

1. Consistence of training for all astronauts
2. Training based on the specific needs in space and not modification of training based on civilian needs
3. No time lost on their relevant skills and knowledge education
4. Ability to add additional information if needed
5. Based on training methods specific for the adult learner
6. Medical skills that provide patient care in space for many conditions otherwise necessitating need for expensive and unnecessary early return form orbit.

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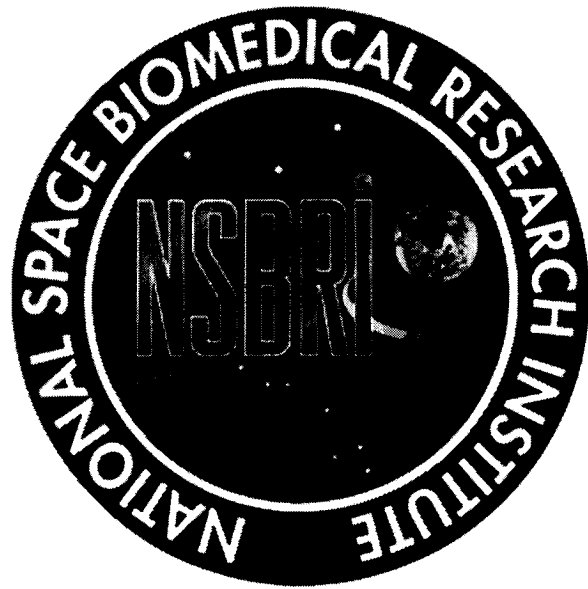
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Appendix J

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



***EDUCATION AND PUBLIC OUTREACH PROGRAM
YEAR 6 - FY 2003***

September 30, 2003

**NSBRI EDUCATION AND PUBLIC OUTREACH PROGRAM
FUNDED PROJECTS**

Team Leader:	MacLeish, M. T.		Morehouse School of Medicine	
Associate Team Leader:	Thomson, W. A.		Baylor	
Gannon, P. J.	PI	Mount Sinai	Defying Gravity: Enduring Life in Space	2
Illman, D. L.	PI	Washington	Northwest Outreach Program on Space Biomedical Research (in Association with <i>Northwest Science and Technology Magazine</i>)	4
Kushmerick, M. J.	CO-I	Washington		
James, R. K.	PI	Texas A&M	National Space Biomedical Research Institute Teacher Academy Project	6
MacLeish, M. T.	PI	Morehouse	Secondary and College Education for the Next Generation of Space Life Scientists	7
Newman, D. J.	PI	MIT	Space Biomedical Sciences and Engineering Curriculum and Outreach Program	9
Merfeld, D.	CO-I	Harvard		
Trotti, G.	CO-I	Trotti and Assoc., Inc.		
Lathan, C.	CO-I	Anthro Tronix		
Smith, R. B.	PI	Rice	Outreach Program for the Professional Development of Students and Teachers on Studies Related to Biomedicine in Outer Space	11
Sognier, M. A.	CO-I	UTMB		
Thomson, W. A.	PI	Baylor	From Outer Space to Inner Space: Sharing NSBRI Progress with the Community	12
Moreno, N. P.	CO-I	Baylor		
Tharp, B. Z.	CO-I	Baylor		

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Patrick J. Gannon, Ph.D.
ORGANIZATION:	Mount Sinai School of Medicine
PROJECT:	Defying Gravity: Enduring Life in Space

Project Executive Summary

Phase one of Defying Gravity involved formulation of perspective-driven scientist, teacher and student partnerships that utilized a stepped mechanism to develop curriculum components within a summer institute: “2001: A Space Biomedicine Research and Education Odyssey.” We recruited teachers and 9th and 10th grade students from top science schools in New York City including Bronx High School of Science, Hunter High School and Stuyvesant High School. Eight module-specific scientist, teacher and student teams helped formulate five common components: 1) pre-lesson/lab activities; 2) formal, inquiry-based lesson plans; 3) scientist research presentation; 4) hands-on laboratory session; and 5) teacher’s guide. Incorporated throughout were factors such as: 1) alignment with National Science Education Standards and Performance Indicators; 2) student interest, incentives and outcomes; 3) mathematical principles; 4) ease of incorporation and utilization by teachers; and 5) cross-team assessment and evaluation mechanisms.

During phase two, beta testing of eight (multi-component; twice-monthly) educational modules was completed at Manhattan Center for Science and Mathematics High School and Life Sciences Secondary School.

Each module consisted of: a) a take home pre-lesson activity; b) a 45-minute classroom lesson [with formal written lesson plan]; c) a 10-15 minute space biomedicine scientist presentation; d) a take home pre-laboratory; and e) a 45-minute, hands-on, inquiry-based, classroom laboratory exercise with data collection, analysis and discussion of findings with a formal written laboratory plan/guide.

The testing, to a group of 35 self-selected 9th grade students at Manhattan Center for Science and Mathematics High School, involved multiple assessments. Defying Gravity had the lowest attrition of any after-school program in the school’s history and the self-selected students who attended did better on New York state formalized examinations than their classmates. Assessment data collected from all levels of participants allowed for reformulation of components for phase three. Module-specific assessment report sheets for student participants and student and teacher curriculum associates were re-formulated and circulated each session. Results were reported back to the Program’s Curriculum Assessment Associate and the Advanced Placement-level Assessment Coordinator for evaluation. Many of these questions are linked to Science Education Standards and Performance Indicators.

Beta-Tested Modules

1. BON-e VOYAGE IN SPACE: If You Don’t Use Them You Lose Them
2. Smells to Mars and Back: When Quality of Life Goes Life Threatening
3. Thinking Big Possibilities: How Far is Safe in Space?

4. Balance Your Life In Microgravity: Countermeasures Rule, OK
5. Silent Communication Around 360 Degrees: How Useful Are Everyday Gestures in Space?
6. Sleep and Performance in Space: Rest Well and Do Well
7. Esprit de corps to Mars and back: Tolerating a tight Space In Deep Space
8. Radiation on earth and in space: Dangers and solutions

Presentation of each module involved:

- Participation of self-selected after-school class of 35 9th grade students (91 percent minority individuals) 29 from Manhattan Center for Science and Mathematics High School and six from Life Sciences Secondary School
- Module-by-module (perspective-based) program evaluation by:
 - Group of four senior (10th and 11th grade) students from Manhattan Center for Science and Mathematics High School
 - Group of six high school biology/math teachers familiar with program
- Non-biased program assessment
- Production of digital video archive

During phase three, four modules are being site tested in teacher's workshops and classrooms locally and nationwide to determine stand-alone potential and practicability of general incorporation within high school curriculum and across grades 8-10. Schools involved include: 1) Hunter College High School; 2) Manhattan Center for Science and Mathematics; 3) Brooklyn Technical High School; 4) Queens Gateway to Health Sciences Secondary School; 5) Bayard Rustin High School for the Humanities; 6) Science Skills Center; and 7) Life Sciences Secondary School. Module-by-module assessments will be obtained from all teachers. Stand-alone site test teachers will be recruited for participation in workshops at the National Science Teachers Association Meeting. We anticipate availability of five-six modules by close of 2003. National Science Teachers Association workshops will be conducted to recruit participants for phase four, a stand-alone teacher-friendly, Web-accessible educational product. Modules are being assessed for inclusion in exhibits at the New York Hall of Science and for Teachers Professional Development within summer workshops of the Department of Education and American Museum of Natural History. They will also be included as workshops at the Teachers Association Meetings (NSTA) driven by goals of the No Child Left Behind Act. We have also initiated an interactive Web site in which stand-alone lesson plans can be downloaded and assessed: <http://www.mssm.edu/defyinggravity/>.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Deborah L. Illman, Ph.D.
ORGANIZATION:	University of Washington - <i>Northwest Science and Technology Magazine</i>
PROJECT:	Northwest Outreach Program on Space Biomedical Research

Project Executive Summary

The overall goals of this outreach project at the University of Washington (UW) are to develop new ways to communicate more effectively with the public about space biomedical science and technology, and to attract bright young minds to careers in this field.

Objectives

1. Develop and disseminate articles on space biomedical research via *Northwest Science & Technology (NWS&T)* Magazine, a regional science publication with a circulation of more than 30,000 in the Pacific Northwest region and beyond, and furthermore, to involve student writers in our science writing curriculum and in development of these articles;
2. Develop/adapt and disseminate special materials on space biomedical research for middle school students and their parents and teachers by means of an insert in *NWS&T*;
3. Improve the ability of scientists and public information officers to communicate with general audiences by developing and delivering a science writing workshop for NSBRI consortium members; and
4. Attract students in the pipeline to careers in space biomedical research by means of a summer experience for high school students in the laboratories of NSBRI projects at the UW.

Accomplishments

1. Magazine Articles on Space Biomedical Research

- Developed the following articles for *Northwest Science & Technology Magazine* on space biomedical topics:
 - “UW Joins Effort to Advance Space Biomedical Technology,” Holli Riebeek, *NWS&T*, Autumn 2001, pp. 6-8.
 - “Flying Higher and Faster: An Interview with Astronaut Susan Helms,” Cover story by Holli Riebeek and Deborah Illman, *NWS&T*, Winter 2002, pp. 14-20.
 - “Great Expectations: Virtual Reality,” Holli Riebeek, *NWS&T*, Spring 2002, pp. 22-28.
 - “Biomedical Studies Should Take Priority on International Space Station: Research on human adaptation to space is likely to benefit terrestrial medicine,” Editorial by Martin J. Kushmerick, *NWS&T*, Spring 2002, p. 58.
- SciScope* insert for middle school students, Spring 2002, by Holli Riebeek.

- Provided support and experiential learning opportunities for a master's-level science writing student, Holli Riebeek, to develop these articles. Ms. Riebeek graduated in June 2002 and is currently serving as technology journalism intern in New York at *IEEE Spectrum*, the flagship publication of the Institute for Electrical and Electronics Engineers.
- Added 4,500 names of middle school science teachers and administrators from WA, OR, ID, MT, and AK to our magazine distribution list to receive these articles and insert.
- Published and disseminated an announcement by Baylor College of Medicine on the NSBRI-funded teaching series "From Outerspace to Innerspace" in the Autumn 2001 issue.
- Published and disseminated full-page color displays about NSBRI in Winter, Spring, and Autumn 2002 issues.
- Technical Communication undergraduate student Sara Causey helped to develop a second issue of *SciScape* on space/biomedical content.
- Provided support for Technical Communication student Marita Graube to develop an article on space exercise.

2. Middle School Insert

- Developed the concept and design for *NWS&T SciScape*, our insert for middle school students, with input from educators and writers with special expertise for this age group.
- The first of two NSBRI-sponsored inserts was published and copies supplied to the outreach team leader.
- Developed an evaluation tool to obtain feedback from users of *SciScape*.

3. Improving Communication with General Audiences

- With funding from the UW College of Engineering for UW School of Communications Ph.D. student Fiona Clark, we completed a content analysis of recent *New York Times* coverage of space-related events and issues. Our objective was to characterize current journalistic practices with regard to space exploration and space biomedical research, and to provide a benchmark against which future developments in coverage can be assessed.

4. Summer Experience for High School Students

- Implemented a recruitment plan in conjunction with Washington Space Grant to identify high school students to work in the laboratories of UW-NSBRI investigators. The plan culminated in the placement of four students in NSBRI-related laboratories at the University of Washington.
- Developed a participation plan for UW-NSBRI investigators to take part in the selection process and tours for 40 high school seniors who are applying for Washington Space Grant scholarships, thereby highlighting topics in space biomedical research for a wider pool of students in the pipeline.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Robert K. James, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT:	NSBRI Teacher Academy Project

Project Executive Summary

Vision

The vision of the National Space Biomedical Research Institute Teacher Academy Project (NSBRI TAP) is to engage middle-level students in the highly motivational study of NSBRI research and long-duration space flight. The project is designed as an avenue to promote improvement in and to contribute to the reform of science education in this country. NSBRI TAP has developed the exciting concept of producing a national cadre of 80 Fellows of the Academy, trained to lead space science workshops in each state of the nation with the focus on NSBRI research. The Teacher Academy is being developed and refined as a model that can be disseminated nationally.

Mission

The mission of NSBRI TAP is to prepare this national cadre of space science teacher-leaders to assist other middle-school teachers to implement NSBRI, space-based science activities in their classrooms. There are about half a million middle-school classrooms in the nation and space-based science provides exciting, creative and inclusive instructional activities that engage middle-level students. The goal is for the Fellows of the Academy to have a significant impact on the space science teaching of their peers and on the learning of students nationwide. Through the joint efforts of NSBRI scientists and Fellows of the Academy, information is being shared to create the vision needed for public support of long-duration space missions.

Impact

The impact of NSBRI TAP on teachers and students is already being felt in thirty-one states. After two years, almost a thousand teachers estimated to serve at least 180,000 students were reached through Academy Teachers' workshops. Sessions, delivered at regional and national conferences by both teacher participants and TAP staff, have been well attended, indicating that NSBRI research is a topic that both interests and motivates teachers and their students. After three years, the project will have recruited a teacher/leader from each state in the nation, Washington, D.C., and Puerto Rico.

Structure and Activities

The first part of the institute is held at Texas A&M University with research contributions from NSBRI scientists on site. The second part is held at Johnson Space Center, Houston, where participants meet with astronauts, NASA scientists and engineers. TAP Fellows from previous years are invited to a meeting held in conjunction with the American Astronautical Society (AAS) Conference where they are provided continuing education about NSBRI research and are supported in their efforts to broaden educational outreach efforts in their home states.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Marlene Y. MacLeish, Ed.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT:	Secondary and College Education for the Next Generation of Space Life Scientists

Project Executive Summary

Mission

The National Space Biomedical Research Institute (NSBRI) Education and Public Outreach Program's (EPOP) mission is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained by the biomedical advances achieved by other NSBRI research teams.

This mission is being accomplished through an integrated array of programs that focus on students and educators at all grade levels, as well as the general public. Specific Team objectives address (1) Teacher Professional Development, (2) Curriculum Development, (3) Science Literacy and Public Awareness and (4) Access and Career Awareness.

The Morehouse School of Medicine's (MSM) program, *Secondary and College Education for the Next Generation of Space Life Scientists*, supports this mission through (1) a Teacher Institute (TI), which is dedicated to the professional development of the minority group science teacher workforce (2) production of secondary level problem/inquiry-based science cases (3) a Summer Research Program to support a pipeline of undergraduate minority group science students interested in research and (4) the maintenance of a film archive to supply design footage for producing curriculum supplements and web-based materials.

Program Impact

MSM-EPOP Year Two activities have reached thousands of students and teachers, created successful partnerships to position NSBRI as a significant contributor to the diversification of the Nation's workforce, and demonstrated NSBRI's capacity to lead international interchanges on the relevance and importance of space based science education. MSM has accomplished, and surpassed, its Year Two objectives despite a disastrous fire and budget reductions. MSM is working with other NSBRI Education and Public Outreach teams to transfer the excitement and real-world relevance of NSBRI research into classrooms and homes through the dissemination of NSBRI curriculum supplements and the professional development of science teachers across the nation.

Future Plans

MSM will use the findings of the first two years of activities to sculpt the development of the following Year Three activities:

1. Conduct a yearlong Teacher Institute for approximately 10 teachers.
2. Maintain and expand partnership opportunities to engage minority group science teachers in professional development activities that incorporate NSBRI education products.
3. Recruit at least six undergraduate students and conduct a 2003 Summer Research Program, including follow-up on a database of 22 students enrolled to date.

4. Complete the final iteration of the problem-based cardiovascular case, *Bobby's Beat*, and disseminate it nationally.
5. Create partnerships to prepare the MSM undergraduate pilot course, *The Human Body in Space*, for Internet dissemination.
6. Continue to develop and implement cost-effective strategies for communicating NSBRI research to local and national audiences.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Dava J. Newman, Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT:	Space Biomedical Sciences and Engineering Curriculum and Outreach Program

Project Executive Summary

Mission

Our mission is to provide the National Space Biomedical Research Institute (NSBRI) with a multi-level Space Life Sciences curriculum, and to excite and educate the public about the wonders of science, engineering and medicine by disseminating knowledge gained in NSBRI research areas. Our project is directly contributing to the need for developing graduate and undergraduate level curriculum, as well as to the need for transfer of advanced space life sciences knowledge into material appropriate for younger students and the general public.

Basic Approach

The team includes academia and two small businesses, both of whom significantly contribute to our outreach program. We are developing and teaching graduate level courses at the Massachusetts Institute of Technology. The curricula are modular and cover eight of the eleven NSBRI research areas, and hence they can be distributed and used in other NSBRI-affiliated universities. The two graduate courses are: “Space Biomedical Engineering and Life Support” (SBE) and “Sensory-Neural Systems: Spatial Orientation from Vestibular End Organs to Behavior and Adaptation” (SNS).

The curriculum and student term projects from the SBE course serve as a starting point for the development of undergraduate-level curriculum and provide the topical areas for outreach at the high school and public outreach levels. We are developing and will teach a freshman course devoted to the same space biomedical topics covered in the graduate-level course. The high school level outreach encompasses Anatomy and Physiology class laboratories that focus on the debilitating effects of long-duration exposure to microgravity on the human body. These are part of a greater engineering design curriculum, entitled “Spacercise,” which challenges students to design the best countermeasure exercise machine for space. For the public, we have completed a conceptual design for the “knowledge station” that allows learners to interact with curricular materials via state-of-the-art information technology and a physical platform that is designed specifically to facilitate human interaction and learning.

Attention to assessment has been paramount in our curricular efforts and learning will be assessed in all our efforts. We have developed two Assessment Guideline Tutorials that give advice to teachers/instructors on 1) effective feedback and 2) learning styles and teaching. A third tutorial is under development and explores the topic of “Questioning” and getting students involved in taking responsibility for their own learning.

Major Accomplishments

We taught the two proposed graduate-level courses, “Space Biomedical Engineering and Life Support” and “Sensory-Neural Systems,” at MIT through the Harvard-MIT Health Sciences and Technology Program. Both courses had NSBRI-affiliated researchers, from several different disciplines, participate as guest or active lecturers.

Development of the undergraduate version of “Space Biomedical Engineering” began with the identification of appropriate level materials as well as a Web site that parallels the graduate site. Extensive discussions and planning with three external academic sites that wish to offer the undergraduate curriculum commenced (Smith College, the Naval Academy, and the University of Maryland). We will continue our efforts with teaching and implementing the course at Smith College in conjunction with their new undergraduate degree in engineering. We are also planning a freshman seminar at MIT.

Three “Spacercise” laboratories are being developed, of which two are scheduled to be piloted. We are currently working to develop the inquiry-based labs for the Anatomy and Physiology classes. Based on discussions with teachers, it was decided that three labs should be developed, each covering one of the following topics: the Skeletal, Muscular, and the Cardiovascular systems. Within each unit, students will gain an understanding of the basic anatomy and physiology of each system as well as an application of the effects of long duration spaceflight on each system.

Three conceptual designs for a “Knowledge Station” were completed and a final concept chosen, namely, the “Knowledge Sphere” design. Visualizations of the Knowledge Sphere, Knowledge Spider, and Knowledge World designs were completed and electronic visualizations and three-dimensional models have been completed for the Knowledge Sphere and Spider concepts.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	Roland B. Smith, Jr., Ed.D.
ORGANIZATION:	Rice University
PROJECT:	Outreach Program for the Professional Development of Students and Teachers on Studies Related to Biomedicine in Outer Space

Project Executive Summary

Our goals are to attract young people to space-related enrichment programs, promoting excellence and innovation in America's science education system, and enhancing the scientific background among teachers, students, their families, and the community as a whole. This is achieved through the programs' two funded components:

- 1) The Academic Development of High School Students (Student Research)
- 2) The Teacher Institute for the Advancement of Space Science Education (Teacher Institute).

Both students and teachers are involved in ongoing space biomedicine research projects conducted at Rice and The University of Texas Medical Branch (UTMB), including those in the areas of i) bone and musculoskeletal loss, ii) integrated human function, iii) immunology, infection, and hematology, iv) neurovestibular adaptation, and v) technology development.

About 12 high-school students conduct laboratory research each summer, taking advantage of the academic environment at both Rice and UTMB. Student interactions include a wide variety of laboratory research in space biomedicine and participation in a series of workshops designed to expose young scientists to experienced researchers. The research experiences are supplemented by field trips designed to motivate students to pursue scientific research as a career.

The Teacher Institute continues throughout the academic year after an intensive two-week session held each June. About 16 secondary teachers enhance their scientific knowledge of space biomedicine through interactive discussions with space biomedical researchers, a one-day, hands-on research experience performing activities in some of the existing NSBRI and NASA educational modules, and special tours of NASA, Johnson Space Center and Space Center Houston. Teachers apply their knowledge toward the design of space biomedicine mini-module/units to be taught in their classrooms during the academic year and refined for publishing on the educational resources Web sites of Rice, UTMB and the NSBRI. Both student and teacher components are monitored and evaluated by an external evaluator.

NSBRI AREA:	Education and Public Outreach
PRINCIPAL INVESTIGATOR:	William A. Thomson, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT:	From Outer Space to Inner Space: Sharing NSBRI Progress with the Community

Project Executive Summary

Baylor College of Medicine is focusing its NSBRI-sponsored outreach activities on elementary and middle school students, teachers and families. These activities include the creation of unique teaching materials, delivery of professional development to teachers by multiple partners, and use of broadcast media to convey general science and health messages to the target populations.

Original Aims

Original project aims at Baylor include:

1. Collaboratively create, evaluate and disseminate interdisciplinary teaching units, based on NSBRI research themes for middle-school students.
2. Improve teacher practice and content knowledge through multiple professional development opportunities conducted in formal and informal educational settings.
3. Develop an online workshop resource for NSBRI scientists to use for outreach to teachers, students and the community-at-large.
4. Create and implement cost-effective models for communicating NSBRI research to local and national populations through television and radio short-format news and newsmagazine stories.

Specific Year Two (2002-2003) objectives and results for Baylor's activities included:

1. *Analyze field test results and reviews by specialists of the Food and Fitness unit, and produce a final version of this teacher guide for national dissemination by institutional members of the Education and Public Outreach Team and other NSBRI partners.* A new NSBRI-funded curricular unit for grades 3-8, *Food and Fitness*, was field tested in spring 2002 and will be published in 2003. Evaluation of the field test produced a large quantity of significant data, related not only to the effectiveness of the unit, but to young students' general understanding (or lack thereof) of the relationship of diet and activity to human fitness. A research article, *A Measure of Knowledge and Misconceptions Regarding Food and Fitness Among Students in Grades 3-7*, was submitted to the *American Journal of Health Education* and is being reviewed. Discussions are under way with a major national science education publisher regarding the development of materials kits for all NSBRI curricular units created by Baylor.
2. *Create, evaluate and disseminate an interdisciplinary, teaching unit (tentatively entitled "Disease and Infection") for middle school students, based on NSBRI research themes.* Baylor is preparing this unit for field testing. In addition, we have conducted teacher professional development activities on topics and materials related to disease and infection.
3. *Improve teacher practice and content knowledge through multiple professional development opportunities conducted in formal and informal educational settings.* During the 2002-2003 funding year, approximately 1,800 teachers, representing about 43,000 students, were reached through Baylor's NSBRI professional development at various venues around the

nation, including Space Center Houston. Field tests results are showing that our NSBRI instructional units are effective, interesting to students and teachers, and simple enough for teachers to use without participating in special training.

4. *Continue to develop and implement cost-effective models for communicating NSBRI research to local and national populations through public media.* We are finding that space life science topics are appealing to the general public when presented via television or radio formats. During Year Two, approximately 1.2 million potential viewers had an opportunity to see NSBRI-focused stories on Houston Public Television, via two appearances by Dr. Nancy Moreno, representing Baylor's NSBRI Education and Public Outreach component. Each of these two, five-minute segments was broadcast at least four times.

Impact of Findings

Through its educational and outreach efforts, Baylor is working to transfer the excitement and real-world applications of NSBRI research into the nation's classrooms and homes. Teachers and students across the United States are using our activities guides to bring NSBRI science to the elementary and middle school level. In addition, the NSBRI-focused professional development activities for elementary and middle school teachers continue at an even greater rate than in the past.

Project evaluation indicates that Baylor's NSBRI efforts have had a significant positive impact in classrooms in Houston and elsewhere across the United States. We are using teacher feedback to continue refining the instructional model upon which we base our teaching materials and professional development activities. In particular, we are working to ensure that our NSBRI-related programs address identified needs of students to develop problem-solving and critical thinking abilities, healthy lifestyles, and language skills within a real world context.

Appendix K

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE



Annual Program Report: *Education and Public Outreach Team*

October 31, 2003

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I. ABSTRACT

The Nation's education needs have been framed by the President's challenge to, "leave no child behind" and the 21st century workplace requirement for a science literate society. Such ambitions require bold vision and strong leadership for systemic change across the educational spectrum. NASA Administrator Sean O'Keefe has taken up this challenge, stating, "Education is part of our [NASA's] core mission." That mission includes the goal, "to inspire the next generation of explorers. . . as only NASA can."

The National Space Biomedical Research Institute (NSBRI) Education and Public Outreach Team has been working to achieve these directives through a coordinated, multi-institutional strategy that helps to meet our nation's education needs. This strategy is designed specifically to assist in educating the next generation of space biomedical researchers and to transfer the medical and biomedical findings of space research to the scientific community, the home and the classroom. The Team's mission is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained by the biomedical advances achieved by other NSBRI Research Teams. This mission currently is being accomplished through an integrated array of programs that focus on students and educators at all grade levels, as well as the general public. The Education and Public Outreach Team develops and implements activities that address the following four major goals:

- Design and conduct a variety of **teacher professional development** programs to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students.
- Develop **curricular materials** that span the educational continuum; are aligned with national science education standards; provide accurate, balanced, effective and inquiry-based instruction; and expand students' understanding of and interest in ongoing NSBRI research.
- Promote **educational access and career awareness** in bioastronautics research among high school and undergraduate students, as well as high school teachers.
- Increase **scientific literacy and public awareness** of the real-life impacts of NSBRI research through media, informal science activities, direct mailings and journal publications.

The Education and Public Outreach Team is comprised of seven primary partners: Baylor College of Medicine (BCM); Massachusetts Institute of Technology (MIT); Morehouse School of Medicine (MSM); Mount Sinai School of Medicine (MSSM); Rice University and The University of Texas Medical Branch (RU/UTMB); Texas A&M University (TAMU); and the University of Washington (UW). Twenty-seven other organizations and institutions—including state public school systems, public television and radio stations, state space grant programs and museums—are working with the Team to promote its mission and to ensure the widest possible dissemination of its products and programs.

The Education and Public Outreach Team is helping NSBRI to address the educational goals set forth by President Bush and Administrator O'Keefe. Hundreds of teachers and thousands of students have benefited from the Team's NSBRI-sponsored programs; and the public has been reached through television and radio news programs and national magazine articles. The Team's ongoing efforts are establishing NSBRI as a leading resource for bringing the excitement and importance of NSBRI space life science research into the nation's classrooms and homes.

II. INTRODUCTION

The mission of the NSBRI Education and Public Outreach Team is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained via the biomedical advances achieved by NSBRI Research Teams. This mission is being accomplished through an integrated array of programs focusing on students and educators at all grade levels, as well as the general public. Team goals are as follows:

- Design and conduct a variety of **teacher professional development** programs to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students.
- Develop **curricular materials** that span the educational continuum; are aligned with national science education standards; provide accurate, balanced, effective and inquiry-based instruction; and expand students' understanding of and interest in ongoing NSBRI research.
- Promote **educational access and career awareness** in bioastronautics research among high school and undergraduate students, as well as high school teachers.
- Increase **scientific literacy and public awareness** of the real-life impacts of NSBRI research through media, informal science activities, direct mailings and journal publications.

III. TEAM STRUCTURE AND DESIGN

The Education and Public Outreach Team is comprised of seven primary partners: Baylor College of Medicine (BCM) in Houston, Texas; Mt. Sinai School of Medicine (MSSM) in New York, New York; Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts; Morehouse School of Medicine (MSM) in Atlanta, Georgia; Rice University and The University of Texas Medical Branch (RU/UTMB) in Houston and Galveston, Texas; Texas A&M University (TAMU) in College Station, Texas; and the University of Washington (UW) in Seattle, Washington.

A number of major organizations and institutions are working with NSBRI's Education and Public Outreach Team. Notable among these partners are (in alphabetical order): Aldine Independent School District, DeKalb County Public Schools, Atlanta Educational Telecommunications Collaborative, Inc., Atlanta University Center, Emory University Center for Behavioral Neuroscience, Fernbank Science Center, the nine Galveston County school districts, Georgia Institute of Technology-SECME Program, Georgia State Partnership for Excellence in Education, Harvard Medical School, Houston Independent School District, Johnson Space Center, New York Public Schools, New York Hall of Science, Space Center Houston, Spelman College, Texas Alliance for Science, Mathematics and Technology, Texas Rural Systemic Initiative, the Texas Statewide Systemic Initiative, and the Washington Space Grant program.

Synergy among individual project goals is achieved through the following four themes: teacher professional development; curriculum development; science literacy and public awareness; and access and career awareness. The following charts show how the teams work with each other.

NSBRI Education and Public Outreach Team Activities, 2002-2003

PI/PROJECT	GOAL ADDRESSED				PHASE I	PHASE II
	Teacher Professional Development	Curriculum Development	Science Literacy and Public Awareness	Career Awareness and Access	Planning Implementation Evaluation	Dissemination
Patrick J. Gannon <i>Defying Gravity: Enduring Life in Space</i>	Science Teacher Summer Workshops, Institutes	9th Grade Curriculum	Museum Exhibits Newsletter and Websites	Museum Exhibits Newsletter and Websites	Project Year 3	
Deborah L. Illman <i>Northwest Outreach Program on Space Biomedical Research</i>			NSBRI Magazine Stories; Science Communications Workshops	NSBRI Magazine Stories; Middle School "SciScape" Inserts	Project Year 3	
Robert James NSBRI <i>Teacher Academy Project</i>	Master NSBRI Teacher Program Teacher Workshops	NSBRI research related, inquiry-based classroom activities	Press releases and coverage of TAP teachers in their home states	TAP teachers interact with 4 NSBRI researchers on A&M campus and with NASA Astronauts and engineers	Project Year 3	NSBRI TAP activities presented at national and state conferences and at local level
Marlene MacLeish <i>Secondary and College Education for the Next Generation of Space Life Scientists</i>	Year-long Residency Program for 17 Secondary School Teachers	Undergraduate Course Problem-based Cases: 5-12 Grade	NSBRI Film Archive	Undergraduate Summer Research Program	Project Year 6	Curriculum Materials Available
Dava J. Newman <i>Space Biomedical Sciences & Engineering Curriculum and Outreach Project</i>		Undergraduate and Graduate Courses; K-12 Materials	Modular Knowledge Stations (Interactive Exhibit)	Modular Knowledge Stations (Interactive Exhibit)	Project Year 3	
Roland B. Smith <i>Outreach Program for Development of Students and Teachers on Studies Related to Biomedicine in Outer Space</i>	Year-long Program for 16 Secondary School Teachers	Teacher-developed Units		Summer Research Experiences for 12 High School Students	Project Year 3	
William A. Thomson <i>From Outer Space to Inner Space: Sharing NSBRI Progress with the Community</i>	Summer Workshops and Workshops at Professional Meetings	Three NSBRI-focused Teacher Units Completed (for Middle School)	Radio and television stories; Space Center Houston Activities	Radio and television stories; Space Center Houston Activities	Project Year 6	Curriculum Materials Available

IV. PROGRAM ACCOMPLISHMENTS, NOVEMBER 2002-OCTOBER 2003

Baylor College of Medicine—From Outer Space to Inner Space: Sharing NSBRI Progress with the Community.

- Conducted 16 NSBRI unit workshops and presentations for 765 educators, representing more than 19,000 students. These activities included presentations at the following meetings.
 - National Association of Biology Teachers (NABT), Cincinnati, OH, November 2, 2002

- Conference for the Advancement of Science Teaching (CAST), El Paso, TX, November 8, 2002
- NASA's Office of Biological and Physical Research Meeting, Missoula, MT, November 19-22, 2002
- Texas Council for Elementary Science Symposium, Austin, TX, January 17, 2003
- National Science Teachers Association (NSTA) Conference, Philadelphia, PA, March 28, 2003
- International Academy of Astronautics (IAA) 14th Humans in Space Symposium, Banff, Calgary, Canada, May 19, 2003
- National Association of Biology Teachers (NABT), Portland, OR, October 9, 2003
- Filled requests from around the US, including Space Center Houston at Johnson Space Center, for more than 1,900 copies of BCM's NSBRI Teacher Guides and more than 1,700 NSBRI classroom posters.
- Incorporated NSBRI activities into other programs:
 - used in a summer professional development program, funded by the National Science Foundation, for 120 lead science teachers in the Houston Independent School District; and
 - implemented in workshops for eight scientists and 16 teachers participating in BCM's Science Education Leadership Fellows program, funded by the Howard Hughes Medical Institute.
- Published BCM's latest NSBRI Teachers Guide, *Food and Fitness*. This third unit in the *From Outer Space to Inner Space* series produced statistically significant increases on pre-post-test student scores related to awareness and knowledge of issues involving cardiovascular fitness, nutrition and the importance of diet and exercise.
- Continued developing the fourth unit in the *From Outer Space to Inner Space* series.
- Wrote an article entitled "A Measure of Knowledge and Misconceptions Regarding Food and Fitness Among Students in Grades 3-7." This article, which discusses BCM's most recently published NSBRI Teacher Guide, *Food and Fitness*, has been accepted, pending revisions, by the journal, *Cell Biology Education*.

Massachusetts Institute of Technology – Space Biomedical Sciences and Engineering Curriculum and Engineering Curriculum and Outreach Project

- Teaching graduate course at MIT: "Space Biomedical Engineering and Life Support" (Fall 2003). <http://paperairplane.mit.edu/16.423J>
- Taught undergraduate course in conjunction with Smith College: "Space Biomedical Engineering and Life Support Systems" (Fall 2002).
- Capstone student project, "Human Performance: Changes in Spatial Orientation and Vestibular Behavior as a Result of Changes in Gravity," flew on the KC-135 (March, 2003).
- Taught graduate course at University of Maryland: "Space Human Factors and Life Support" (Spring 2003).
- Completed physical design and construction of "Knowledge Station," to promote public awareness of NSBRI and NASA educational outreach efforts (November 2003). Software implementation is in process.
- Developed two high school level anatomy and physiology laboratory modules that focus on space biomedical sciences and engineering principles, particularly the Skeletal and Muscular Systems.
- Outreach to several high school groups (with approximate number of students)
 - MIT's High School Scholar Program (24 students)

- GetSSET (Sport Science, Engineering, and Technology) Academy (15 female students)
- Exploring Engineering at the University of Maryland (E2@UMD) (2 X 25 female students)
- Charlestown Boys & Girls Club (15 inner city or minority students)

Morehouse School of Medicine—*Educating the Next Generation of Space Life Scientists.*
The MSM Teacher Institute

- Admitted 17 science teachers, representing 2,048 students, to a yearlong Teacher Institute.
- Completed nine teacher professional development modules, listed below.
 - Teachers Teaching With Technology
 - Creating Twenty-First Century Classrooms
 - Integrating Technology into Classrooms — *Flex Microscope, Sensors, Weather Station; Smart Medical Systems for the Journey to Mars (NSBRI)*
 - Problem-based Learning for Greater Understanding
 - Principles of Field Testing
 - Function and Structure of the Heart — Pig Heart Dissection (FSC Laboratory)
 - The Human Body in Space on Earth
 - Muscles and Bones
 - Leadership for Transformation — *Challenger Disaster; Lean on Me (Film)*
- OBPR Workshop: *Leading Great Research in Space*, November 17-22, Missoula, Montana
Completed two workshops and two presentations for 150 teachers.
 - Staffed NSBRI's Education and Public Outreach exhibit and disseminated approximately 500 NSBRI products.
- Regional Academic Service Learning Conference Center, May 1, 2003, Atlanta, GA
 - Conducted two leadership workshops for 80 teachers.
 - Disseminated 250 NSBRI-EPOP products.
- 27th SECME Summer Institute: July 13-24, 2003, Tennessee State University, TN
 - Conducted five modules for approximately 180 teachers from across the Nation: *The Journey to Mars, Striking a Happy Balance, Sleep and Circadian Rhythms, The Cardiovascular System in Space, and Pig Heart Dissection Laboratory session*
- NSBRI-JSC Summer Internship Banquet: July 21, 2003, Houston, Texas
- Co-sponsored film premier, *Partners of the Heart*, February 8-9, 2003, Atlanta, GA.
 - Teacher Institute teachers used materials to design inserts for the problem-based case, *Bobby's Beat*.
- TAMU-TAP: Presented the Leadership Lecture, July 23, 2003, College Station, TX.
- 17th Annual Symposium, Career Opportunities in Biomedical Sciences, April 15-8, 2003, Nashville, TN
 - Exhibited MSM-EPOP materials, recruited Summer Research Program students, disseminated 500 products.
- 14th IAA Humans in Space Symposium, May 18-22, 2003, Banff, Canada

- Submitted paper, National Space Biomedical Research Institute Education and Public Outreach: Communicating Bioastronautics Research to the Public Students and Families, to journal, *Acta Aeronautica*, 2004.
- Chaired International Symposium Education and Public Outreach session.
- NASA Awareness Days, Atlanta University Center, October 14-16, 2003, Atlanta, GA
 - Staffed NSBRI exhibit for approximately 200 students.
 - Conducted tour for NASA, CODE E
 - Welcomed NASA Director, Sean O’Keefe to AUC

Summer Research Program

- Four undergraduate students completed a ten-week intensive research program including: circadian biology laboratory research; weekly journal club presentations; school-wide poster session/final oral presentation; science writing workshop; core laboratory skills and ethics training.
- Updated longitudinal database on 22 past students for program outcome measurements.
- Conducted Exit interviews and evaluation for summer students.

Mt. Sinai School of Medicine—*Defying Gravity: Enduring Life in Space*

- Presented DG program at Canadian Space Agency’s, 14th IAA Humans in Space Symposium, Banff (report of proceedings posted to DG web site).
- Submitted 32-page article to journal *Acta Astronautica*.
- Conducted four hour Defying Gravity workshop at NSBRI-EPO Teachers Academy Project. Multiple teachers conducted Defying Gravity lessons... ongoing site testing.
- Conducted preliminary assessment of extended CROSS-GRADE utilization of DG curriculum at Ida Strauss elementary school in NYC (PS-198): from original 9th - 10th grades to 4th - 5th grades.
- Public Media Exposure (feature articles about Defying Gravity): The Island Current (monthly NYC newspaper) - “Sailing the Seas of Space” (Circulation 10,000)
Big Apple Parent (monthly NYC newspaper) - “Bringing Space Travel Research Into the Classroom” (Circulation 77,000).
- Life Sciences High school in NYC dedicated entire week to Defying Gravity program (350 students and five math/biology teachers). Concluded with final session at Mount Sinai with guest speakers and student presentations.
- DG Program student Sarah Walter awarded GOLD STAR for formulating new Defying Gravity ACHIEVEMENT PATCH PROGRAM for Girl Scouts of USA.
- DG website upgraded extensively and being used extensively nationwide to download curriculum modules. www.defyinggravity.net
- Plans are underway to solidify Defying Gravity collaboration with the Boy and Girl Scouts of America. NASA HQ (Biological & Physical Research - Bryan Morris & Bonnie McClain) were willing to work with NSBRI-Defying Gravity program to bring this to fruition.

Rice University/University of Texas Medical Branch—*Space Science Education Teacher Institute for the Advancement of Space Science Education*

- Enrolled 16 high school teachers in a yearlong residence to develop space biomedicine curriculum modules.
- Completed a special tour of NASA Johnson Space and Center and University of Texas Medical Branch laboratories to support curriculum content and design.

- Teachers participated in a regional science conference (September 20, 2003) and exhibited curriculum exercises developed during the two-week summer program to an audience of 200 teachers.
- Continual field-testing of instructional activities developed during the two-week professional development program is in progress (July 2003 to present).
-

High School Student Research Program

- Twelve students completed a summer research internship program, including authentic laboratory research, weekly research seminars series conducted by faculty, field trips to Johnson Space Center/NASA, and a brown bag lunch discussion of career opportunities.
- Twelve faculty members from Rice University and UTMB involved in NSBRI related research areas served as preceptors for students working in their laboratories (one faculty member per student). In addition, students worked closely with laboratory research assistants and post-docs in all assigned laboratories.
- All students from the Rice and UTMB campuses attended a tour of Professor James Smalley's Rice University Nanotechnology laboratories and a presentation on Nanotechnology by Dr. Kristen Kulinowski, Executive Director for Education and Public Policy, Rice University Center for Biological and Environmental Nanotechnology (CBEN).
- Closing Poster Session and Reception held at UTMB August 1, 2003, where students showcased the focus and results of their summer research projects.

Texas A&M University—Teacher Academy Program (TAP)

- Completed an 11-day summer institute at Texas A&M University and Johnson Space Center, Houston (July 19-30) for 27 participants from 24 states plus Washington DC and Puerto Rico.
- TAP III, 2003-2004, included a teacher from Puerto Rico who has agreed to translate the NSBRI TAP inquiry-based classroom activities into Spanish.
- Two NSBRI TAP II teachers, Jennifer Linrud-Sinsel (KS) and Teri Rowland (WY), have made it through to the final round of selection for the Educator Astronaut Program and each spent a week at Johnson Space Center for interviews.
- Project Coordinator worked in the schools of participating teachers in the states of KS, MN, WI, MT, WY, SD, TN, AR, PA, TX, OK and OH to support them during the year. He also taught NSBRI Space Science for a day at the Kickapoo Indian Nations School in Kansas.
- Project Director and Coordinator delivered a paper at the Conference of the National Association for Research in Science Teaching (NARST) in Philadelphia, March 23-26, 2003. The paper was entitled "NSBRI TAP - A Staff Development Strategy with a Built-in Multiplier Effect."
- NSBRI TAP II teachers taught 2,607 students in their schools, of whom 717 are African-American, 76 are Asian, 378 are Hispanic, and 38 are Native American.
- NSBRI TAP II teachers gave workshops to an additional 1,413 science teachers in their states to increase the impact of the project.
- Project staff presented NSBRI TAP workshops at: the International Space Station Educators' Conference, Houston, TX, February 2003; Youth Symposium on the Texas A&M campus, TX March 2003; NSTA Philadelphia, PA, April 2003
- Revised the NSBRI-TAP teacher training resource book for further testing by 2003-4 participants.
- Alternative avenues to promote NSBRI research:
 - TAP II teacher Mellisa Duncan (KS) has just been appointed Lead Flight Director at the new Challenger Center in St. Louis, MO.

- TAP II teacher Jennifer Linrud-Sinsel has been made an adjunct professor teaching one-credit aerospace education courses at Oklahoma University.
- Tap II teacher Dr. George Stewart will teach Aerospace Physiology at the University of Alaska's Aviation Campus.
- TAP I teacher Joy Reeves has been appointed "Teacher on Loan" at the Museum of Science and Industry, Chicago, Illinois.
- 25 teachers from TAP I and from TAP II will join with TAP III to attend a mini-conference in Houston, TX, November 15-19, 2003. The focus is to develop even more successful NSBRI TAP workshops looking at components such as funding sources, advertising and securing college credit.

The University of Washington—Northwest Outreach Program on Space Biomedical Research

- Developed articles on space and space biomedical research for publication in *Northwest Science & Technology (NWS&T)* magazine, disseminated to 30,000 readers in the Northwest and beyond.
- Printed NSBRI's display ad in each issue of the magazine published during the project.
- Developed the second of two inserts for young readers, called *SciScape*, on space biomedical research and disseminated it to 30,000 readers, including 5,000 secondary school teachers, throughout the greater Pacific Northwest.
- Recruited four students who completed the 2003 high school summer research program, bringing the total to date of eight summer students.
- Initiated planning and development of a science communication workshop for NSBRI teams to be delivered during the coming year.
- Research article on media coverage of space published in the September 2003 issue of the journal *Science Communication*.

Implications of Current Accomplishments

The National Space Biomedical Research Institute (NSBRI) Education and Public Outreach Team is addressing NASA's educational mission and increasing the visibility of NSBRI research through teacher professional development, curriculum development, science literacy/public awareness and career awareness and access. An impressive array of programs has been planned and implemented. From November 2002 through October 2003, NSBRI teacher professional development reached more than 3,000 teachers nationally and up to 75,000 students through 60 teacher summer institutes at Baylor College of Medicine and Mt Sinai School of Medicine, year-long teacher residency programs at Morehouse School of Medicine, Rice University/University of Texas Medical Branch at Galveston, and Texas A&M University. These NSBRI teams also presented NSBRI educational workshops at national meetings and in informal science settings, including Space Center Houston.

The NSBRI Education and Public Outreach Team certainly has become more directly involved with NASA's OBPR educational enterprise during the 2002-3 program year. For example, Education and Public Outreach Team members presented NSBRI-funded educational materials at the OBPR meeting in Missoula, Montana in November 2002; they also attended meetings of NSBRI team leaders in Washington, DC and at Johnson Space Center to share progress of the Education and Public Outreach Team and to participate in developing OPBR's Education and Outreach Strategic Plan. Moreover, NASA is using educational materials developed by NSBRI's Education and Public Outreach Team for workshops it is conducting, as handouts at national meetings, and on NASA-produced television programs.

NSBRI's Education and Public Outreach Team has demonstrated considerable success in sharing the importance of space life science education and its relevance to the missions of NSBRI and NASA. However, much needs to be accomplished. NSBRI leadership plans to reorganize the Education and Public Outreach effort should result in even greater impacts in the future.

Based upon experiences to date, it is clear that the NSBRI Education and Public Outreach Team should: (1) continue to develop greater levels of partnership and alignment with NASA's educational activities, particularly those of OBPR and Bioastronautics at JSC; (2) possess a thorough understanding of OBPR's new "Core Values and Organizing Objectives" for all programs, as well as an understanding of NASA's Office of Education (Code N) criteria for the evaluation of NASA's education programs; (3) increase emphasis on dissemination of materials already developed by the NSBRI Education and Public Outreach Team; (4) create a "brand name" name for "Education and Public Outreach" among the nation's schools and teachers; (5) expand use of the Internet to reach ever larger (world wide) audiences for educational materials and information; and (6) generate greater levels of professional publications relating to NSBRI education and outreach activities.



Marlene Y. MacLeish, Ed.D.
Leader
NSBRI Education and Public Outreach Team

October 23, 2003
Date

Appendix L

NSBRI Teacher Workshops/Conference/Participants

October 1, 2002-September 30, 2003

Lead NSBRI Institution	Name of Workshop	Date	No. of Teachers
Rice University/ University of Texas Medical Branch	Rice/UTMB Curriculum Development Follow-up Workshop	1/18/03	18
	Rice/UTMB Teacher Professional Development Program (10-Day)	6/2-13/03	15
	UTMB Regional Science Teacher's Conference (workshop presented)	9/19/03	15
Texas A&M University	Teacher Academy Program Workshop (2-week)	7/19-30/03	27
TAP II, 2002-2003 TAP Teacher to teacher. Examples of types of workshops/outreach, by state	Nancy Houtkooper (MN): Spring MNSTA Conference, St. Cloud, MN * Space Science Conference at Science Museum of MN in St. Paul	3/14/03	11
	Alicia Baturoni (WI): Space Education Initiatives, summer training Wisconsin Society of Science Teachers Convention, Madison, Wisconsin in-service	Summer 2003 3/11/03	137 25
	Tracy Davis (OH): Cleveland Clinic, OH (2 days)	7/25-26/03	28
	Linda Beebe (MI): Wayne County Regional Education Service Agency Detroit Urban Systemic Initiative, NW High School, Detroit, MI (2 days)	11/08/02	26
	Pam Whiffen (AZ): Arizona, school district Penn State Conference for Science Teachers	8/19-20/03 7/01/03	90 20
	Lisa Horton (WA): Seattle Museum of Flight two teacher sessions "Life as an Astronaut" Museum of Flight Seattle, WA	Summer 2003	16
	Jen Linrud (KS): Cosmosphere Hutchinson KS (2 days)	11/22-23/02	22
	Lauren Allwein (CO): Denver Museum space workshop "Journey to Mars"	2/22/03	25
	Euclid Middle School, CO: Hands on workshop. NSBRI research.	4/22/03	12
	Debby Salter (WA): University of Washington Fairbanks, AK Alaska Space Grant Consortium	12/07/02 2/15/03	9 16
	Shirley Green (MT): Living in Space, Lewis & Clark MS Billings	1/21/03	6
	Juliet Sisk (TN): Countermeasures for long duration space flight, Motlow Junior College	11/04/02 12/14/02	10 10

	Steve Hove (NV): Space Flight Physiology, Reno (2 days) "Marsopolis": Sparks NV	2/07-08/03 2/22/03	27 13
	Jennifer Milligan (AR): "What's up in Space?" Hot Springs	2/24/03	35
	Teri Rowland (WY): Biomedical Effects of Space Flight, Sheridan	11/23/02	7
TAP I, 2001-2002. Workshops given by Fellows of the Teacher Academy beyond their year in the program.	Lois Eppich (KS): Delta Kappa Gamma State Convention	4/04/03	50
	NCEA Catholic Educators, St. Louis Kansas Association of Teachers of Science Convention	4/21/03 4/25-27/03	200 50
	Penny Glackman (PA): Program for Gifted Youth, Lehigh University	Summer 2003	30
	Sandy Armstrong (AL): Wiregrass Math and Science Consortium at Troy State University, Dothan, AL	Summer 2003	50
	Jan French (OH): Classroom activities at Cincinnati Museum Center	Mar/Apr 2003	20
	Joy Reeves (IL): "Museum Partners" Chicago Science Museum	4/26/03	25
Mount Sinai School of Medicine	NSBRI Defying Gravity Workshop	7/23-24/03	27
	NSBRI Defying Gravity Workshop (5-Day)	3/24-27/03	5
Morehouse School of Medicine	MSM Teacher Institute: Nine Teacher Professional Development Modules	Nov 2002-- Feb 2003	17
	OBPR Workshop: Leading Great Research in Space, Missoula, MT	11/17-22/02	150
	14 th Annual Humans in Space Symposium, Banff, Canada	5/20/03	35
	Regional Academic Service Learning Conference, Atlanta, GA	5/1/03	80
	Four Workshops: SECME 27 th Annual Institute at Tennessee State University	7/13-24/03	180
Baylor College of Medicine	Make and Take Workshop presented at the Metropolitan Association of Teachers of Science, Houston, TX	11/2/02	150
	Workshop presented at National Association of Biology Teachers Annual Meeting, Cincinnati, OH	11/2/02	30
	Workshop presented at Conference for the Advancement of Science Teaching, Science Teachers Association of Texas, Austin, TX	11/8/02	50
	Workshop, meeting of NASA Office of Biological and Physical Research, Missoula, MT	11/19/02	40
	Workshop presented at the International Space Science Conference, Houston, TX	1/7/03	20

Workshop at the Texas Council for Elementary Science Symposium, Austin, TX	1/17/03	45
Workshop presented at Space Center of Houston	2/1/03	26
Workshop presented at the Annual meeting of the National Science Teacher's Association, Philadelphia, PA	3/28/03	30
Make and Take Workshop presented at the Annual Meeting of the National Science Teachers Association, Philadelphia, PA	3/29/03	150
14 th Annual Humans in Space Symposium, Banff, Canada	5/20/03	35
Workshop presented at the Bioastronautics/College of the Mainland Professional Development Conference, Johnson Space Center, Houston, TX	6/18/03	20
Workshop presented for Clear Lake teachers at Johnson Space Center	6/20/03	10
Workshop at the TAP II conference at Texas A&M	7/22/03	22
Baylor Science Leadership Program (NIH)	7/14/03	132
My Health My World Institute (NIH)	8/8/03	25
Totals	53 Events	2,324 Teachers

NSBRI Education and Public Outreach Conference Data

October 1, 2002-September 30, 2003

CONFERENCES	DATE	# Attendees
World Space Congress	October 14-19, 2002	1,300
National Association of Biology Teachers, Cincinnati, OH	November 2, 2002	300
NASA OBPR Strategic Planning Conference, DC	November 17-19, 2002	20
NASA Workshops, Missoula, Montana	November 17-22, 2002	300
Black History Month: <i>Partner's of the Hearts</i> , Atlanta, GA	February 8-9, 2003	400
City-Wide Curriculum Symposium	March 27, 2003	71
National Science Teachers Association Conference, Philadelphia, PA	March 28, 2003	150
17 th Annual Symposium: Career Opportunities in Biomedical Sciences	April 15-18, 2003	1,100
14 th IAA Human In Space Symposium, Banff, Canada	May 18-22, 2003	300
Preliminary Assessment: Cross-Grade Utilization of DG Curriculum	June 2-3, 2003	74
Closing Community Ceremony High School Program, Galveston, TX	August 1, 2003	80
Closing (Parents/Community) Summer Research Program, GA	August 14, 2003	60
TOTAL		4,155

Appendix M

NSBRI 2003 SUMMER INTERNSHIP PROGRAM

INTERN	SUPERVISOR	ASSIGNMENT	DATES
Andrew Abercromby University of Houston	Dr. Bill Paloski/JSC 281-244-5315	Bldg. 37/Room 152A Neurosciences Laboratory	5/19/03 - 8/22/03
Anton Aboukhalil McGill University	Dr. Mark Shelhamer/JHU 410-614-6302	Department of Otolaryngology and Biomedical Engineering	5/27/03 - 8/31/03
Eric Feiveson Rice University	Dr. Philip Foster/JSC 281-483-7267	Bldg. 37/Room 1082 Environmental Physiology Laboratory/JSC Pulmonary Medicine/Baylor	6/2/03 - 8/8/03
Katharine Forth University of Houston	Dr. Bill Paloski/JSC 281-244-5315	Bldg. 37/Room 152A Neurosciences Laboratory	5/27/03 - 8/29/03
Milan Lombardi Vassar College	Dr. Janice Meck/JSC 281-244-5405	Bldg. 266 Cardiovascular Lab	5/27/03 - 8/1/03
Vickie Maul Georgia Institute of Technology	Dr. Linda Shackelford/JSC 281-483-7100	Bldg. 261 Bone and Mineral Laboratory	5/19/03 - 8/8/03
James Rexroth University of Texas at Austin	Dr. Todd Schlegel/JSC 281-483-9643	Bldg. 37/Room 1060 Neurosciences Laboratory	6/9/03 - 8/8/03
Waralee Sattam University of Texas at Austin	Dr. Todd Schlegel/JSC 281-483-9643	Bldg. 37/Room 1060 Neurosciences Laboratory	6/9/03 - 8/15/03
Audrey Schaffer Massachusetts Institute of Technology	Dr. Brian Peacock/JSC 281-483-7131	Bldg. 15/Room 260C Habitability and Human Factors	6/2/03 - 8/12/03
Madhurita Sengupta University of Texas at Austin	Dr. Todd Schlegel/JSC 281-483-9643	Bldg. 37/Room 1060 Neurosciences Laboratory	6/9/03 - 8/15/03
Justin Seret Rensselaer Polytechnic Institute	Dr. Linda Shackelford/JSC 281-483-7100	Bldg. 261 Bone and Mineral Laboratory	5/19/03 - 8/1/03
Leah Zidon Truman State University	Dr. Brian Peacock/JSC 281-483-7131	Bldg. 15/Room 260C Habitability and Human Factors	6/9/03 - 8/12/03

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August 9th 2003

Dear Dr. Sutton,

I feel my 3 months in the Neurosciences Laboratory at JSC has provided me a valuable insight into life as a researcher with NASA. Having previously been fortunate enough to experience a year of working in the engineering directorate at JSC, I had some idea of the opportunities and challenges that might arise during my internship and I feel that my previous experiences at JSC stood me in good stead and allowed me to get involved in the projects quickly. The process of getting a computer and an email account took some time, but in fact took less time than I expected, thanks to Elisa Allen and Jan Cook.

The first project to which I was assigned initially struck me as an interesting and achievable project given the time constraints of working only 20 hours per week for 12 weeks. The project involved a cooperative effort between researchers at JSC and their Russian counterparts. In brief, the premise of the project was that Russian and US neuroscience researchers currently use two different protocols to evaluate neurovestibular functions before and after spaceflight. One involves perturbing subjects using a moving forceplate (Equitest posture platform) on which they stand (lower body perturbation), while the other perturbs the subjects by pushing them in the chest (upper body perturbation). Ground reaction force data and kinematic data are gathered during both protocols. A draft manuscript had already been written and some data had been gathered. My assignment was to gather some more data and work on the manuscript.

The first indication that the project may not be useful was the suggestion that the data had not initially been gathered with the intention of writing a paper. The data existed and attempts were being made to write a paper around that data. After reading the manuscript a couple of times, it appeared to me that the only question being addressed was of minimal significance. Specifically, it was suggested that the different perturbation techniques lead to different kinematic results. Upper body chest pushes cause the chest to move before the legs while moving the feet using a translating forceplate causes the feet to move before the chest. While this finding is relevant in terms of determining what is actually being measured by each protocol (determining the relative contribution of proprioceptors, vestibular system, vision etc in maintaining balance) I did not feel it merited the writing of a paper in itself. Following an extensive literature review, I concluded that the only useful questions that could be addressed with the data had already been comprehensively addressed in several other studies and that the finding of different kinematic parameters between the two conditions did not merit the writing of a paper.

I discussed these matters with Dr. Paloski and several colleagues and it was agreed that no further work should be performed on the study given the findings of the literature

review. Despite the outcome of this initial project, I feel I learned a lot in terms of the theory of computerised dynamic posturography (CDP), its functions, how it works and how it is used in the Neurosciences lab. I was able to critically evaluate the data and the draft manuscript and make recommendations based on existing knowledge and knowledge gained through the literature search – skills I have learned in previous academic projects and expect to use frequently in the future.

The project on which I spent the majority of my summer was very valuable to me personally in terms of practical experience of using hardware and software in the laboratory and going through the experimental design process from concept through to data capture. Analysis of the data will not be performed before the end of my internship. The idea behind the project was to conduct a pilot study in which I would use existing laboratory hardware i.e. an Equitest posture platform, an OptoTrak, and electromyography, to measure ankle impedance in standing and seated subjects. The rationale is that ankle impedance may provide a measure of lower limb muscle tone. Muscle tone has been observed to be affected by spaceflight, with hypertonia being observed qualitatively during standing and hypotonia during sitting. The aim is to develop a quantitative technique to measure standing muscle tone using existing hardware.

Based on another literature review and existing knowledge from research performed in the lab at University of Houston, I wrote an experimental protocol. Limitations of the hardware meant that the protocol was not exactly as I would have liked, but there was sufficient evidence in the literature that I might get useful data even with the hardware constraints.

Perhaps the most enjoyable and most useful days of my internship were those spent in the lab configuring the equipment and software for data collection. Having recently acquired an Optotrak in the lab at the University of Houston, I was particularly happy to have the opportunity to experiment with it and get a basic understanding of its capabilities and operation. I look forward to applying this knowledge in future studies.

The process of awaiting approval to gather data on test subjects took considerably longer than hoped though not much longer than expected! During this time I wrote step-by-step procedures for the protocol, a task which was not particularly enjoyable but one that I wanted to do in case the protocol proves to be useful beyond my internship, as I hope it will.

Getting everything in place to collect data was slightly more challenging than I expected, having never played such a major role in a physiological study. There are so many elements that must come together at the same time before even a single data point can be plotted. And naturally, several important elements were somewhat out of my control! While I thought my organizational skills were fairly good before this summer, I realize that there is still plenty of scope for improvement!

However, the planets eventually aligned and data was collected. The value of the study will remain unclear until the data has been analyzed, which a colleague is working on now. On a personal level, the value is already clear for me to see. I am glad that this summer has been challenging and I feel that, for the most part, I have dealt with the challenges well.

I feel very fortunate to have been given the opportunity to spend my summer working for NSBRI. As a British citizen, I am very well aware of the fact that it is often a difficult task to employ and badge non-US citizens and I am extremely appreciative of the efforts of the staff at NSBRI, Dr. Paloski and Elisa Allen in the Neurosciences Laboratory for making this internship possible. I feel I was made very welcome and everyone was very generous with their time, which I appreciate greatly. The opportunity to work with people of the calibre of those employed in the Neurosciences Lab and with NSBRI is a privilege and an extremely valuable experience for me.

I hope to spend more time working with NSBRI in the future, both during and after my studies at University of Houston.

Many thanks,

Andrew Abercromby

Anton Aboukhalil Internship Report

As an NSBRI summer intern, I have had the chance to spend 14 weeks at the Johns Hopkins School of Medicine, working under Dr. Mark Shelhamer's supervision in the Otolaryngology/Head & Neck Surgery department. I only had hopes of participating in such a program as a foreign national. Moreover, due to my undergraduate status, the employment process at Hopkins was difficult. Despite all these seemingly insurmountable bureaucratic complexities, the Baylor HR team – along with Ms Kathy Major and Mr. Jim Cooper – has successfully coordinated the process with the Hopkins HR team – comprised of Ms Patricia Rusche, Jeanne Liesemer and many others. I cannot say how grateful I am for their collective efforts and for the considerable time devoted to make this summer internship happen. I will always remember their help and consideration to me with much happiness and appreciation.

Indeed, what an adventure it was to arrive there! I thus could not let this research internship pass without fully appreciating every single day and making the most out of it. Since the very first instant I stepped into the lab, I felt the spirit of the members and the atmosphere of the research environment. I genuinely believe it was a great match for me. I met Faisal Karmali, a 3rd year PhD student in Biomedical Engineering, who had just slept in the lab that night. Of course, it was Memorial Day, but since we were Canadian, we could not use the holiday as a pretext to not start working. So he began by training me on the video-oculography system and on the analysis software. Since then, my stint has been one of the most extraordinary and profound learning journeys of my life.

With Faisal supervising my work, I had the chance to design and build an instrumentation frame to measure eye movements in parabolic flight aboard NASA's KC-135. We needed the capability of investigating (at least) saccades, vergence, LVOR, and AVOR (and when subjects were sitting straight up or lying supine on the plane's floor). Various design issues were taken into consideration, namely:

- a) Cost
- b) Robustness to withstand accelerations of the order of 10g's
- c) Flexibility to make future modifications to the frame
- d) Dimensions to accommodate various sizes of subjects
- e) Level of comfort for the subjects allowing lengthy experiments

We produced three different designs. I opted for the one I thought was the sturdiest. However, Faisal pointed out to me the advantages of having a flexible system permitting future alteration. Trying to optimize our decision, we agreed on a hybrid structure that was highly adjustable. In the end, I am glad that we chose this option. After ordering the parts and building the frame we realized that we could not attach the frame to the plane's floor (according to the 20" square pattern of floor attachment holes); we needed to widen the assembly by at least 1". Had we followed my original suggestion, we would have needed to reorder numerous pieces; however, with our chosen hybrid design, only three additional aluminum bars have been ordered and the situation has been corrected. Unlike my previous course projects, I realized that practical engineering problems have various potential solutions. It was important for me to develop the ability to work on such problems, evaluate advantages and disadvantages of the available options, consider tradeoffs, and finally implement the optimal or near-optimal solution, all this having the cost factor in mind (~\$1,500). At the end of this month, Dr. Shelhamer and Faisal will use this instrumentation frame when conducting their experiments on board of the 'Vomit Comet'. I am truly excited to receive their feedback.

I also spent a few days of work with the research engineer, Dale Roberts. He explained to me in detail his design of the wireless coil prototype currently under development. At present, the most accurate way to measure eye movements is the scleral search coil method. In short, it is a sort of rubber contact lens with a copper coil embedded on it. By moving the eyes in alternating magnetic fields, alternating voltages are induced in the wire. With proper signal processing, the vertical, horizontal and torsional movements can be accurately determined. The new proposed system can dispose of the wires protruding from the lens and the eyelids, reduce subject discomfort, maintain the original accuracy and bandwidth, and most importantly allow a variety of new experiments to be conducted. Unfortunately, due to time constraints, I did not have the chance to work on it. Nevertheless, I believe it would be interesting to study other potential ways of measuring signals and removing noise. Of course, one can wait and average a signal over time. However, companies in the Mixed-Signal industry (for whom saving every fraction of a second is crucial as they test millions of say Intel chips per month, trying to reach the market before their competitors) use frequency domain filtering. They use the IFFT to separate frequency components from one another. I

find the idea neat and a very powerful filter can be obtained¹. This suggestion might increase the speed and repeatability of measurements and is worthwhile investigating.

Finally, because of the availability of the NSBRI program, I have had the chance to do real science and actively participate in new and exciting funded research. Being on a team, and working in the long-term goal of improving human adaptation to gravitational changes was truly exciting. I was assigned the task of examining the effects of ‘consolidation’ on ‘context-specific adaptation’ (CSA) of saccades. After reading Dr. Shelhamer’s work on the subject, I remarked that CSA has not been quite effective with *vertical eye position* as a context cue. I thus proposed to use a resting period to perhaps improve saccade adaptation in this particular case. Dr. Shelhamer was very open to the ideas and suggestions I put forward; I felt that my viewpoints were important to him. That kept me motivated to keep learning and working with greater enthusiasm. Subsequently, I was trained to use the lab equipment and to measure eye movements with electro-oculograms. A few preliminary experiments were initially conducted to gain familiarization with the laboratory environment and to perfect the experimental procedure. Then, I had the chance to independently run experiments on subjects, analyze the data on MATLAB, and write code for the mathematical processing. Along the way, Dr. Shelhamer guided me, gave me credit for my contributions, and treated me as a graduate student; indeed, it was both enlightening and beneficial for my education to have a glimpse of the graduate student research life that I hope to pursue.

So far, the results show that, in many subjects, a one-minute time gap improves the extent of adaptation and the speed in attaining it. The research work I accomplished this summer has potential for publication and could help in answering one of the NSBRI questions on “Context-Specificity and Other Approaches to Neurovestibular Adaptation”: Does a rest interval between stimuli promote adaptive consolidation?² I feel lucky but excited about this finding.

¹ M. Burns and G. W. Roberts, An Introduction to Mixed-Signal IC Test and Measurement, Oxford University Press, New York, USA 2000.

² Oman, C. M., “NSBRI Neurovestibular Adaptation Team Strategic Plan.” 2003
< <http://www.nsbri.org/Research/StrategicPlans/Neuro2003.pdf> >

Beyond the work in the lab, I was also given the opportunity to attend Dr. Zee's Friday teaching sessions. These were fascinating lectures where I learned a great deal about the circuitry of the brain involved in the control of the oculomotor system.

In short, in this summer experience, I have gained a more practical understanding of engineering design, but, most importantly, I experienced first-hand the realities of how scientific research is conducted in this field. I can sincerely say that conducting research as an NSBRI intern under Dr. Shelhamer's supervision has been by far the most interesting and valuable summer experience I have had thus far. On the whole, I learned that research requires a great deal of perseverance, originality, and late evenings in the lab. Reading scientific papers and understanding their content, especially in neurophysiology, has been an enormous challenge to overcome. Working on problems where no one really knows the answer, asking questions, and trying to think differently have been intellectually stimulating activities. This memorable stay at Hopkins will definitely remain an asset to me in my future research undertakings.

As stated in my application, my original future goals were to pursue graduate school at MIT's Man Vehicle Lab (MVL), if the chance is given to me. Then, I was aiming to actively participate in the human space flight program and collaborate on related international projects. I believe it is important to impress upon you how much this research experience helped me consolidate my motivation and commitment to pursue these career objectives. I feel more determined and ready to follow this dream and turn it into reality. As a graduate student or a researcher, if possible, I would like to contribute to the future NSBRI neurovestibular team's efforts in finding and developing countermeasures against the vestibular concerns caused by prolonged space flight. I must recognize that NSBRI has helped in opening doors to carry on potential further research at Hopkins or might even facilitate collaboration between the latter and the MVL to investigate context-specific adaptation in short-radius centrifuge, or to investigate some countermeasures for the space shuttle landing vertigo problem in a more direct way.

At Hopkins, I have learned a great deal from the first until my very last moment. I seriously mean it. On the last day, I attended the lab's yearly grand event, 'The Crab Party'. There, Dr. Shelhamer taught me the complex technique of eating crabs. As anything else in life, it was a lot of work, but it was worth learning it!

Internship Report

For my internship, I worked at Johnson Space Center with Dr. Phillip Foster for two months. Dr. Foster has been doing research in environmental physiology at Baylor College of Medicine. I helped with his current project, which is to find a way for astronauts to avoid decompression sickness (DCS) when they perform an extra-vehicular activity (EVA). DCS is caused by the nitrogen bubbles that form in certain critical areas of body tissue because of reduced pressure in the astronaut's spacesuits. In order to reduce the degree of bubble formation and growth, the astronauts attempt to purge as much nitrogen as possible before they begin the EVA. They do this through a "pre-breathe" preparation protocol. Typically lasting about three hours, the protocols require the astronauts to exercise and breathe various gas mixtures, including pure oxygen. During the protocol, experimenters control the type and amount of exercise the astronauts perform, as well as the partial pressures of the gases in the atmosphere they breathe. Researchers are currently studying several types of protocols to determine which one best removes the nitrogen while still meeting time and other logistical constraints.

My project involves a computer program that first simulates the nitrogen removal process that occurs during four different protocols. Then, the program uses the amount of nitrogen remaining at the onset of the EVA to estimate the average bubble volume and bubble radius in the arms and legs of the astronauts every ten minutes during the EVA.

Besides the attributes of the protocol, the program uses three other parameters in its computation - the astronaut's maximum oxygen consumption rate, the fraction of the astronaut's inspired oxygen that reaches the astronaut's tissues, and a statistical

parameter, beta, which represents the intensity of a Poisson process, by which the bubbles form.

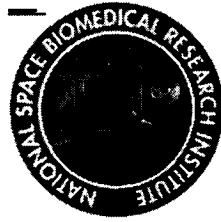
On my first day, I was given a working version of the program, written in Mathematica. My job was to improve the program by making it more flexible, easier to use, and easier to understand. Over the past two months, I accomplished this by doing the following:

- Rewrote the program to improve its readability. The volume of code was reduced from 4 MB to 25 KB without sacrificing functionality.
- Increased the program's efficiency. The time the program requires to run one simulation was reduced from 15 minutes to about 40 seconds.
- Allowed the program to run with any protocol, not just the four protocols the program was originally designed for.
- A new program, called GenData (written in C++), was created to streamline the running of the bubble growth simulation. It's many features include:
 - Running the simulation of the protocol and the bubble growth thousands of times, looping the chosen protocol, the astronaut's maximum oxygen consumption rate, the fraction of the astronaut's inspired oxygen that reaches the astronaut's tissues, the Poisson parameter beta, and the part of the astronaut's body that is simulated.
 - Using Mathematica to perform a non-linear regression to approximate the bubble volume vs. time function with a log normal function

- Allowing the user to quickly create customized profiles and simulate their effects

In the process of writing the program, I learned a great deal. In addition to becoming familiar with Mathematica itself, I also had to research the procedure, known as mathlink, for communicating with Mathematica from within a C++ program. In addition, in creating the GenData program, I had to research many aspects of Windows programming, such as dealing with the clipboard, adding special features in the program's dialog boxes, and keeping the program from freezing while waiting for Mathematica to solve a differential equation. In addition, to understand the workings of the original program, I had to refer to two of Dr. Foster's papers on the subject (one has already been published and one is currently being reviewed) to determine how the equations in the program worked together.

In the future, plan to study computer science on a formal basis for the first time, building on what I have accomplished. I also hope to come back and work for NSBRI next year.



NSBRI Summer 2003 Internship Report

Name: Katharine Forth

Placement: NASA/JSC Neurosciences Lab

Advisor: Bill Paloski

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My primary project during the summer was to manage and analyse the Baltimore Longitudinal Study of Ageing (BLSA) dataset with the intention of extracting some publishable data.

Work completed:

The major tasks completed at NASA included:

1. learning Matlab to a functional level
2. reviewing the literature surrounding time to contact, the equitest system, and postural strategies.
3. mining the data for possible trends
4. verifying calculation of the data analysis
5. reanalyzing data with a new script
6. investigating a number of possible statistical methods to approach the data
7. creating a results section
8. writing a paper (minus methods section) to be submitted for publishing

The BLSA data is a massive dataset, which includes 188 participants each with two sessions of six conditions with 3 trials. Each trial consisted of 2060 data points. Needless to say, this was the largest database I have attempted to extract useful information.

Despite the wealth of data collected, previous attempts to tackle the data had failed due to the size of the database and the time that was required to manage it. Furthermore, the neuroscience lab had moved on to an improved data collection technique for their clinical astronaut testing, but struggled to move forwards with the new protocol, knowing ageing trends from the BLSA will provide guidance. Consequently, it seemed any headway made on the BLSA data was much appreciated and applicable to current and future Equitest posture testing.

The final paper covered a comparison between the EQ score, generated by the Equitest system, and the new time to contact measure, across a comprehensive range of ages (20-90yrs). The rationale behind choosing this topic was the need to establish time to contact as a measure, and demonstrate its increased sensitivity to postural deficits, which the EQ score fails to represent. This is particularly important as the EQ score is used by medical clinics and for clinical astronaut testing to identify disorders and/ or deficits in balancing. The paper also provides a base protocol and foundation from which further time to contact posturography studies can be based. For example, the postural strategies adopted during each trial of the BLSA could be time synched to the time to contact, providing an appropriate parameter that will likely predict the change in strategy.

My immediate plan for the future is to publish the paper written during this summer internship. In addition, my other plans include aiming to finish my PhD by summer 2004. After finishing my PhD, I shall be looking to find a postdoctoral position, preferably within the context of Space life sciences.

I thoroughly enjoyed my experience with NSBRI and JSC neuroscience lab, and look forward to maintaining and developing the relationships built during this summer.

Thank you very much!

Milan Lombardi

Cardiovascular Lab Summer Research Internship

While working in the cardiovascular lab under Dr. Janice Meck I was given the responsibility of performing two retrospective analyses using data collected from the astronauts. Both studies were geared toward a better understanding of space flight induced orthostatic intolerance. The first study involved the astronaut's use of phenergan and phendex (phenergan with dextroamphetamine) in flight to combat motion sickness, the most common ailment among astronauts. Given the antihistaminic properties of phenergan and the related CNS stimulant properties of phendex, the lab was very interested in discovering what effects these common drugs had on the astronaut's cardiovascular system. In short, I collected and analyzed this data, to the extent of a publishable nature. Due to a lack of statistical significance caused mainly by the limited number of subjects, any articles stemming from this data will have to await the addition of further subjects.

The second study was aimed at decoding the differences, if any, between stand and tilt testing methodologies used to detect orthostatic intolerance. This study is of particular importance to the lab as well as all labs that participate in upright tilt testing. In the cardiovascular lab, a number of studies have been published that combine data from both methodologies, which switched from stand to tilt testing after STS-81. Since this variable exists and its statistical significance was yet to be determined, the results from this study were relevant to all researchers combining data from these two

methodologies. In this study, a statistical significance was established and I am hoping the results will be published in an upcoming article.

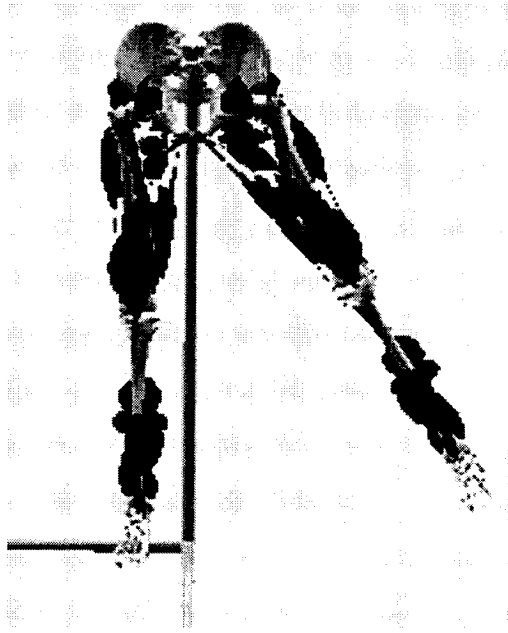
At the end of my term with the lab, I gave a comprehensive one-hour presentation that outlined these two projects from start to finish. The purpose of this presentation was to educate the lab in regards to my findings as well as to better educate myself in the skill of presenting intellectual scientific material. It was a success and I have been invited back to work on future research projects.

The experience to work at the Johnson Space Center in the cardiovascular lab is one that I had not thought possible as an undergraduate. I am very grateful that I was fortunate enough to be given this opportunity. During my ten-week internship I learned a great deal about cardiovascular physiology and the mechanics of conducting clinical research. I am pleased that during my stay I was able to achieve a personal benefit as well as make a positive contribution to the lab.

Looking into the future it is my aspiration to gain entrance into medical school and perhaps practice aerospace medicine. During the summer I was able to meet with some of the flight surgeons at NASA and discuss with them how they got where they are today and what career path decisions I should be making if I wanted to end up there myself. This internship also provided me with insight into the various research disciplines needed for manned space exploration. The human space program is a very romantic ideal for our time, one which I support with great enthusiasm, and I am honored and thankful to have been a part of it in my own small way. Thank you.

Vickie Maul

Georgia Institute of Technology



National Space Biomedical Research
Institute

Bone and Mineral Lab

Summer 2003

I was assigned to Dr. Linda Shackelford in the Bone and Mineral Laboratory at JSC for my NSBRI summer internship. I quickly learned that astronauts experience bone loss due to a decrease in muscle activity in microgravity. Therefore, the general purpose of the Bone and Mineral Lab is to reduce the loss of bone mineral density (BMD) and the probability of forming osteoporosis during long duration space flights. One of their most recent reports was on a 17-week continuous bed rest study, which is the best way to imitate microgravity at long periods of time. It consisted of an exercise group, 5 male and 4 female, and a control group, 13 male and 5 female. The exercise group followed a strict exercise regiment on the Horizontal Exercise Machine (HEM) for each week. Dual Energy X-Ray Absorptiometry (DEXA) and Magnetic Resonance Imaging (MRI) scans were taken pre and post bed rest for each test subject to measure any difference in BMD.

The results were astounding. The resistive exercises showed an overall positive effect, especially in the pelvis, lumbar spine, and heel. This study also proved that lower body muscle mass and volume had no significant change in the exercise group. Calcium balance, which is one way to detect bone loss, was preserved in the exercise subject, but negative in the control group. Additionally, the greater trochanter had the greatest amount of bone loss in the exercise subjects. Thus, even with exercise, the trochanter is the most difficult area to protect.

With this information, Justin Seret and I decided to conduct a detailed study by describing and calculating the muscle loading across the greater trochanter during specific exercises. Those exercises include a hip abduction and a single-legged heel raise with a shallow squat. One way to determine the muscle forces during the exercises was to model them with a software program. After much research, AnyBody was decided to be the best option for a number of reasons. The program has realistic human models, can simulate human movement, allows analysis including muscle and joint forces throughout the movement, and can remove gravity while adding external forces. Since it can also import CAD files into the model, we first assembled the Horizontal Exercise Machine in SolidWorks.

After realizing that programming in AnyBody is not the most simplest, we decided to split the project up. Justin concentrated on the heel raise with the squat while I focused on the hip abduction. I had to make certain decisions and assumptions

concerning the hip abduction exercise before I could get started. First, the resistive force was applied at the ankle and remains perpendicular to the ankle throughout the motion. Second, the motion is created by the gluteus minimus and gluteus medius muscles only. Third, the muscle forces will be measured at 15, 30, and 45 degrees. Finally, the resistive load is the max average load by the exercise subjects.

First I used a simple 2D static model of the exercise with the hip abduction muscle 70 degrees to the horizontal at anatomical position. A resistive load of 65 lbs (289 N) produced a muscle force of 738.85 lbs (3283.8 N) at 15 degrees, 709.3 lbs (3152 N) at 30 degrees, and 729.8 lbs (3243.7 N) at 45 degrees. There is a dilemma, however, when trying to compare these results with the model because the model has six (three gluteus minimus and 3 gluteus medius) hip abduction muscles. They have the same insertion point on the trochanter but dissimilar ending points on the pelvis. Therefore, I decided to take the force vectors of each muscle, which I obtained from force graphs and position outputs, and added them into one vector. I was then able to calculate the magnitude of the three force vectors at 15, 30, and 45 degrees. With the same resistive load of 65 lbs (289 N), a muscle force of 482.8 lbs (2145.7 N) at 15 degrees, 447.6 lbs (1989.4 N) at 30 degrees, and 449.1 lbs (1996 N) at 45 degrees.

In comparing these results, the model reported a smaller hip abductor force at all three angles. The difference between the muscle loads was an average of 266 lbs (1183 N). Despite these variations, they both showed the least muscle load occurs at 30 degrees and the greatest at 15 degrees. One possible reason for these dissimilarities is that the 2D static model was very simplified. Also, AnyBody is programmed to minimize the maximum muscle stress. In addition, combining six muscle forces into a single force is not the most accurate. Moreover, the human body is far too complex for a computer program to perfectly depict it.

Despite the obvious advantages to AnyBody, it does have some disadvantages. First of all, it does not illustrate the external forces, which would allow assurance that the correct resistance force is being applied. It would also help for displaying and presenting the models. Secondly, AnyBody is a code based interface. Most of the time spent on this program was just trying to learn the code and how to manipulate it for this specific project. The code is based off of C++, but unfortunately I have not had experience with

it. Of course, AnyBody is only in its second stage of development so many of these problems will probably be fixed. Also, the program will most likely become more accurate with its results through further testing.

The results achieved through the software program and the simple 2-D model may not exactly represent the gluteus medius and minimus muscle forces during the hip abduction exercise, but it presents a simple, yet vital theory. Since muscle loading stimulates bone growth, then exercises that directly target a specific bone region will help in preventing bone loss in that area. The greater trochanter still experienced a loss in bone mineral density with these exercises. That bone loss in the trochanter, however, is possibly much greater in astronauts and cosmonauts who just use a bike and a treadmill for their exercises. Basically, resistive exercises can target bone areas that need more concentration and loading, so they should be added to the routine. It may even help reduce the time spent on the treadmill and bicycle.

This internship has been an incredible opportunity that allowed me to experience an engineering job within the heart of the space industry. I have always wanted to work at Johnson Space Center and be part of the community that keeps the human space program alive. Even though Columbia was tragic, it seems to have brought everyone together and to strive for a challenging goal. I am positive the American space program will soon send humans back into space. As far as working in the biomedical research area, it was definitely a learning experience. Even though I started with a limited knowledge of the human body, I left with an in depth understanding of the body's dynamics and its importance on bone mineral density. The willingness to explain and answer questions by Dr. Shackelford, John Dewitt and Scott Smith was exceedingly the reason I was able to learn so much. Even though I did enjoy my project tremendously, I have decided to continue in aerospace engineering since I am a 3rd year already. I am planning on returning to Johnson Space Center next summer in the Engineering Directorate to also get a taste of my major. In addition, I plan to attend graduate school directly after graduation. My experience next summer hopefully will help determine whether I remain in aerospace or switch to biomedical engineering. My overall main interest is the human space program, so either way I know I will end up back at Johnson Space Center or other NASA centers. And who knows, one day I may be able to look

down on Earth from the International Space Station or Mars and remember how much I appreciate my first internship, with the National Space Biomedical Research Institute, that got me in the door.

James Rexroth

How an Internship Should Be

When my friends found out that I would be working at the National Aeronautics and Space Administration (NASA) this past summer, they teased me – about going to the moon and building rockets, as well as about other misconceptions associated with the space industry. In reality, however, not many students get the opportunity to engage in such a wonderful experience. As an intern at the NASA Johnson Space Center, I worked for Dr. Todd Schlegel, M.D., in the Neurosciences Laboratory, on his seemingly endless 12-lead PC-based electrocardiogram (ECG) project. The work I performed throughout my internship consisted of writing and debugging programs for Dr. Schlegel's ECG data analysis application.

This project was based on the collection of ECG data through the use of *CARDIAX*, software developed by IMED Co. Ltd. Once data is collected, it must be analyzed. This is done through the use of several C language-based data analysis applications. I primarily worked on applications grouped under the Heart Rate Variability (HRV) application, whose mainframe was developed by one of Dr. Schlegel's team members using *LabWindows CVI*. More particularly, I was responsible for developing the baroreflex sensitivity/controlled breathing, Poincare ellipse, and Fast-Fourier Transformation applications.

My very first task was to write a program that measured baroreflex sensitivity. I was first assigned several medical journal articles to read, in order to understand the concept of baroreflex sensitivity and its significance to heart rate variability. Baroreflex sensitivity is the observed response time it takes for one's brain to speed up or slow down his or her heart rate. It is generally difficult to measure without knowing the patients' blood pressure, which an ECG cannot measure. After reviewing several articles, Dr. Schlegel found a method which could measure baroreflex sensitivity using controlled breathing at 0.1 Hz (one breath every ten

seconds), which creates a sinusoidal R-R interval wave pattern. Given the R-R intervals of an ECG recording, I wrote code that calculated and displayed the mean, median, minimum, and maximum of each R-R interval excursion (i. e. the peak to trough “distance” of the sine wave pattern produced by the 0.1 Hz breathing) and a cumulative root-mean-square estimate of the excursions onto a user interface. Moreover, I also wrote the code for this function to be executed properly. To accomplish this, I had to first make sure the user wanted baroreflex sensitivity calculated through controlled breathing. This was done by using a toggle switch with on/off values – the function was executed when the switch was turned on. Then, I had to make sure that enough heartbeats were collected to execute the function and calculate values properly.

My second project involved the Poincare plot. These plots are derived by plotting an R-R interval ($R-R(n)$) on the x-axis against the following R-R interval ($R-R(n+1)$) on the y-axis. In clinical medicine, the shape of a Poincare plot can reveal certain cardiac disorders, such as myocardial ischemia and parasympathetic nervous activity. However, not all of the data points should be considered – the standard that is used is 66% of the plots. I was assigned to write a function that displayed an ellipse over the appropriate amount of data. Calculating the ellipse is achieved through the measured standard deviations of the data points, which are also the axes of the ellipse. My code plotted the ellipse within the Poincare plot, as well as displayed the standard deviations (axes) on the interface.

The final task that I was assigned was to implement the Fast-Fourier Transform and display the power spectrum of the given data set. The power spectrum plots the frequency in Hertz on the x-axis versus the power in milliseconds squared on the y-axis. The frequencies are generally divided into domains of very low frequency, low frequency, and high frequency. I wrote the program to make these user-defined with standard defaults (VLF: 0 – 0.04, LF: 0.04 –

0.15, HF: 0.15 – 0.5) so that the doctors may specify them as they wish. In addition to displaying the graph, I also calculated and displayed the total power as well as the power and peak frequency in each domain.

In the lab I worked with two other students, Mae Sattam and Madhurita Sengupta. Mae specialized in analyzing the data while Madhurita wrote programs for the HRV application as well. Madhurita and I combined all of our functions along with another team member's, Dr. Carl Greco, together in one program. We also created a new user interface for the HRV application.

This past summer was very memorable and educationally significant for me. I was able to improve my programming skills and become proficient in working as a team. Interning at NASA really is an amazing experience that should never be passed up. This year I am continuing my Bachelors in Computer Engineering at the University of Texas at Austin. I plan to pursue a Masters in Business Administration after graduation. My internship was inspiring in my search for a career. I would enjoy being able to have a similar job involving the tasks I performed at NASA.

Summer Report

By: Waralee Sattam

This summer, I worked in the neurosciences laboratory at Johnson Space Center with Dr. Todd Schlegel, who specializes in cardiology. In his laboratory, my fellow interns and I worked on a project with the main goal of developing and improving software called High Frequency (HF) QRS, which works along side a real-time PC-based electrocardiogram (ECG). With hospitals and clinics using this software, they can better diagnosis people coming in with complaints of chest pain more efficiently and effectively.

The other interns worked on writing code in C programming language to build more applications for the software; whereas, I analyzed data of previous patients in different hospitals in the Houston and Galveston area. This data consisted of measurements from the ECG signals; if I were to find that a person's QT variability index to have a value greater than -1 , there would be a small or no risk of having coronary artery disease. With the program that I wrote on Excel to aid for a more resourceful analysis, I calculated the specificity and sensitivity of the test that was used to attain those measurements in future hopes of writing a medical paper to inform the public of the software. Additionally, I studied numerous medical papers on beat-to-beat QT interval variability in order to familiarize myself with what the figures signify.

Dr. Schlegel and I also visited The University of Texas Medical Branch in Galveston and various hospitals in the Texas Medical Center in Downtown Houston to collect data and encourage doctors and physicians to participate in Dr. Schlegel's study of using the PC-based ECG with HF QRS so he can have more supportive statistics for a

paper. Towards the end of my summer term, I wrote a program in C language to compute approximate entropy with heart rate and RR intervals.

I believe that one of the best experiences I have had this summer has been walking around in the hospitals and seeing first-hand how research is done. I realize now that it is not a simple task, and a career of this sort would require tremendous patience and persistence into meeting financial needs and collecting enough data. However, I still find it a very intriguing career, and in the upcoming years, I still want to continue to pave my way to medical school to become a doctor or researcher. As of now, cardiology has made a noteworthy influence as to what field I would like to specialize in.

Being able to work at Johnson Space Center was incredible everyday. I took advantage of many of the lectures and tours they provided for all of the students working there for the summer. I toured through all of the neurosciences laboratories and saw the equipment they used to help astronauts after their long-duration flights regain their posture and balance. I learned a lot about motion sickness and how eye movements play a significant part in that issue. In the end, meeting Gene Kranz, the Apollo 13 flight director, was unquestionably one of the most memorable events this year, inspiring me to be a leader and fighter for the exploration and further research in space.

The analysis and programs that I made played a role in the project as a whole to design more sophisticated and practical technology for the medical community in regards to cardiology and even the everyday use of the normal twelve-lead ECG machine. During my first couple of days in the lab, I was overwhelmed by foreign medical terminology, programming, and even working with the Excel application; however, by the end of the month, medical papers were an easy read for me and Excel is as simple as

writing in Microsoft Notepad. The larger challenge of programming still remains, but not as significantly as it had been in the beginning of my college career. In the surroundings of such clever individuals at NASA, I have learned well to improvise and think on my feet in search for better solutions for any single problem. I feel incredibly proud and honored to be given this opportunity and to be a part of an extraordinary team of pioneers to breakthrough in the areas of medicine and research.

Audrey Schaffer

Summer Intern Exit Report
National Space Biomedical Research Institute
August 7, 2003

Internship dates: June 2 through August 11, 2003
Mentor: Dr. J. Brian Peacock
Habitability and Human Factors Office (SF3), NASA Johnson Space Center

During my internship, I worked on the development of a habitability assessment tool with Dr. J. Brian Peacock at NASA Johnson Space Center. This tool will generate a holistic assessment of the habitability of a designed system (i.e. a spacecraft). To generate this assessment, the tool will take numerical inputs from the system design (such as interior volume, temperature), assess each one's contribution to habitability, and generate an overall habitability assessment.

My summer work involved identifying those factors of the system design that contribute to habitability. To identify these factors I interviewed a number of experts working in the Habitability and Environmental Factors Office at NASA/JSC. From those interviews, I generated a series of conceptmaps to describe each particular area of expertise. Each concept in the web of concept maps will eventually be an input value for the tool.

My work most directly relates to the development of the habitability assessment tool. The concept maps I have created are the foundation of the habitability assessment project because the tool will eventually take inputs from those areas that I have identified.

In a larger context, the development of this tool relates to the work of the whole Habitability and Environmental Factors Office because of its utility to easily assess the habitability of a system. Currently, a series of requirements, titled the Man-Systems Integration Standards 3000 (a several volume document), must be met when creating engineering designs in order to ensure that a system is

habitable. For example, an engineer designing a piece of equipment must check each requirement while he is designing to be sure that his design values meet the standards. The habitability assessment project aims to simplify this process by allowing an engineer to instead input the design values for his system into the tool, and then automatically determine how habitable that system is, without needing to check each requirement.

The habitability assessment tool will also allow non-technical personnel (such as managers) to quickly assess the overall habitability of a system or set of subsystems. This functionality will allow anyone to make trade-off decisions, without detailed knowledge of engineering design. This application is potentially useful for work at all levels of manned system design, and can be used in other analogue contexts as well (such as ships).

In my view, the most exciting aspects of my work this summer were my interactions with habitability experts. In interviewing and knowledge mapping the person's area of expertise, I was able to learn about their field in depth. At the start of the summer I was not sure what it meant to work in "habitability," and this internship experience allowed me to discover both broadly and specifically what the field entails.

At this point in time, I am unsure of my plans for the future. However, my experiences this summer did help me decide that the next step in my life path will be graduate school in some area of human factors or cognitive systems. I do not know which university I would like to attend, or even what specific field I would like to research. But I do know that I will not be continuing into Aerospace Engineering (my undergraduate degree), but instead will pursue something different.

I was able to make these decisions mostly because of the experience I've gained this summer. Through the NSBRI's generous support, in addition to my work at JSC I was able to travel to the Institute for Human and Machine Cognition (www.ihmc.us), located in Pensacola, Florida. Though the purpose of my trip was to improve my concept mapping techniques to improve my project, simultaneously I was able to learn from scientists there what the field of cognitive systems involved. That experience, combined with a summer of learning about Human Factors and Habitability at JSC, gave me a very good taste of the fields I will pursue.

Thank you again to the NSBRI for supporting me this summer. I had a very enjoyable and exciting experience.

Madhurita Sengupta

My Wonderful Summer at NASA

Ever since I can remember, I have always had an interest in the space program. Reading astronomy books, sitting outside on my lawn and looking up at the stars and identifying constellations was the norm when I was growing up. I can still recall visiting Houston from my previous home in Louisiana and immediately asking my parents to take me to Space Center Houston. Something about the idea of going to space and other planets and learning about our universe was thoroughly intriguing.

So when in high school I found out about an opportunity to participate in a National Aeronautics and Space Administration (NASA) sponsored program, called the Texas Aerospace Scholars Program, I applied without hesitation. After spending a week at Johnson Space Center, I completely fell in love with everything NASA and was determined to get more career experience there. During my senior year in high school, I participated in the Gifted & Talented Mentorship Program, which allowed me to work alongside a mentor in my career field of choice. I chose to work with an aerospace engineer at Johnson Space Center, in the Advanced Mission Design Branch under the Engineering Directorate. It was an enlightening experience that convinced me that I wanted to get even more hands on experience at NASA.

At the end of the summer, before I left for Austin, Texas, to attend The University of Texas, I spoke with Dr. Todd Schlegel, as I had an interest in biomedical based research that also incorporated my major at school, electrical engineering. Dr. Schlegel gave me a synopsis of the Electrocardiograph (ECG) research he was conducting at the time. I became interested in learning more, and Dr. Schlegel recommended that I apply

for the National Space and Biomedical Research Institute's (NSBRI) summer internship program and work for him on his ECG research. Without a moment's hesitation, I applied for the program, and after a few months of waiting, I received my acceptance letter.

I began work in the Neurosciences Laboratory at Johnson Space Center in the beginning of June. I was given an overview of the kinds of projects I would be completing. Myself and another NSBRI intern, James Rexroth, were to develop (programming and debugging) subapplications for the pre-existing Heart Rate Variability (HRV) mainframe, created by a colleague of Dr. Schlegel's. The data analysis methods under the HRV mainframe range from calculating Detrended Fluctuation to calculating Turbulence and Baroreflex Sensitivity, using controlled breathing, to calculating Approximate Entropy. These applications use data collected through CARDIAX, a 12-lead PC-based ECG software system, developed by IMED, Ltd. (Budapest, Hungary), which allows physicians to playback and view ECG recordings. James and I were assigned to develop the aforementioned data analysis applications, as well as a few others. Mae Sattam, the third NSBRI intern in our office, was responsible for researching data analysis methods related to HRV and analyzing samples of data using some of these methods.

After becoming acquainted with the software program (LabWindows CVI) I would be using to do my projects, I began working on my first project - Detrended Fluctuation Analysis, or DFA for short. DFA used a set of RR intervals to calculate sets of points, whose slope was significant in diagnosing certain cardiac ailments. Unfortunately, since DFA had to be calculated using all RR intervals - from the very first

one to the most recent one - it was time consuming, as it had to be calculated each time a new RR interval was acquired. The points calculated in DFA are divided into two sets - Alpha 1 and Alpha 2. After reviewing several medical journal articles on this data analysis method, it was decided that Alpha 1 would range from an x-axis value of $\log_{10}4$ to an x-axis value of $\log_{10}11$. This allowed for exactly eight points. Alpha 2 ranged from $\log_{10}11$ to the most recent point calculated using the RR intervals. I first had to determine the slopes of both Alpha 1 and Alpha 2. Once that was done, I plotted the points, as well as the linear regression lines, which was calculated using all of the points in each Alpha 1 and Alpha 2. This method of calculating linear regression, however, later proved to be troublesome - troubleshooting of the errors generated because of this method will be discussed later. It was also necessary to display the Alpha 1 and 2 values in digital displays. The last step I had to complete in order to complete this project was to execute the function only when enough heart beats were acquired by CARDIAX. Alpha 1 was first calculated after forty-four heart beats were collected, while Alpha 2 was first calculated after eighty-eight heart beats were collected.

The next project I was assigned was to create trend plots for certain analysis methods used in HRV - namely, DFA, Approximate Entropy (AE), Fast Fourier Transforms (FFT), and Time Domain (TD). These trend plots were put in three categories - cumulative, short term, and long term. Cumulative trend plots were simply a plot of various parameters calculated in each of the analysis methods mentioned above. These parameters included: Alpha 1 and Alpha 2 (DFA), ApEn (AE), Very Low Frequency, Low Frequency, High Frequency, and Total Power (FFT), and Mean and Median RR Intervals, as well as a few other very basic calculations related to cardiac

activity (TD). I plotted these parameters on their specific graphs each time the values were calculated. The short term and long term trend plots, however, were calculated a bit differently. User controls were available so that the user could specify how many beats he/she wanted in a "sliding window." The value specified by the user for the sliding window was used to calculate each respective parameter using that many beats. For example, if a user specified 256 beats for the sliding window on the short term trend plot for Alpha 1 and Alpha 2 in DFA, Alpha 1 and Alpha 2 would be calculated for beats 1 through 256, then again for beats 2 through 257, then again for beats 3 through 258, etc. Once these values were calculated, they had to be plotted on a strip chart. The x-axis for each plot had to be adjusted depending on what the value of the sliding window was specified as, as well as depending on what the user specified the length of the x-axis as (this value was used only for the short and long term trend plots). For instance, if the sliding window for a cumulative trend plot was set to 500 beats, the x-axis for the cumulative plot would have to be from 0 to 500 for the first 500 beats. However, for a long term trend plot with a sliding window of 4000, the x-axis would start at 4000 and go to 4000 added to the value that the user specified for the length of the long term trend plots.

As expected, these tasks kept me quite busy - through a significant portion of the summer. In addition to these tasks, however, I also created the user interfaces for each data analysis application, as well as renovated the mainframe. Once these were completed, I ran data files through CARDIAX, in order to debug any problems that may have come up. These errors included the FFT trend plots not plotting correctly, the Turbulence application not working every time the program was run, and translating the

Approximate Entropy application from a static program to a program which calculated values in real time. These errors were fixed eventually, but other small errors came up as well. The main problem I found in debugging the errors was that many times it was incredibly difficult to find exactly what the error was. For many of the errors that did come up, I found that finding the exact cause took more time than actually fixing it. This is the reason I chose to extend my stay at the Neurosciences Laboratory for three extra days. I finally finished all of my tasks and debugged as many problems as I could identify.

This experience was an incredibly eye-opening one. I was given the opportunity to use the knowledge I gained during my first year at school to contribute to a project whose applications and significance to diagnosing cardiac ailments are unbelievable. As for the future, I currently plan on attending the University of Texas at Austin, majoring in Electrical Engineering, as a cooperative education student at NASA Johnson Space Center. Upon graduation, I plan on pursuing a degree in graduate studies. This internship has helped me obtain an even greater understanding in the career of engineering, as related to the space industry - a love of mine. I worked alongside some of the most phenomenal individuals, who taught me the value of teamwork, work ethic, and efficiency in work performance. I hope to use this knowledge, as well as the skills that I have acquired through this experience, in my future career.

Justin Seret

NSBRI 2003 Summer Project Intern Report

Background

Over the course of the summer, I participated in a variety of different activities in the Bone and Mineral Lab at the NASA Johnson Space Center. The main role of the Bone and Mineral Lab is to research the mechanisms involved in the bone loss that occurs during extended space flight, and the countermeasures that may prevent it. The lab often uses bed rest experiments to simulate microgravity. This is because, in either case, there is not nearly as much demand on the musculoskeletal system as there is when one stands and walks regularly on Earth. Most of the work that (fellow NSBRI intern) Vicki Maul and I did was based on a bed rest study that was completed by the lab in 2001.

In the study, 27 participants were placed in horizontal bed rest for 17 weeks. Eighteen of the subjects were part of a control group, which did no exercise. The remaining 9 subjects were assigned into an experimental group that performed various resistive exercises on a weight lifting machine called the "Horizontal Exercise Machine" (HEM). The HEM was specially designed at NASA-JSC for this study to allow the subjects to remain supine (lying down) during their exercises. Our supervisor, Dr. Linda Shackelford, and several of her colleagues compared the changes in bone mineral density (BMD), serum and urinary markers of bone metabolism, and calcium balance obtained before, during, and after bed rest. It was shown through the study that there was significantly less bone loss in the experimental group. Our objective was to analyze some of the exercises performed from an engineering perspective, to find out how the muscle forces that were produced during the exercises were related to the decreases in bone loss.

Experience

During the first couple of weeks, we spent most of our time researching the report that described the study, entitled “Resistive Exercise as an Effective Countermeasure to Disuse Induced Bone Loss.” This helped us to determine the regions of the body in which risk for bone loss was most severe – the lumbar spine and the greater trochanter of the hip. We decided to focus on the greater trochanter region of the femur for our analysis. We also spent time learning about the anatomy and biomechanics of the hip joint. We found out that the greater trochanter is basically used as a “lever arm” by the gluteal muscles at the hip to abduct the legs. This allows us to do things like stand on one leg without falling to the side. In the experiment, the muscles were important for the “hip abduction” and “one-legged squat” exercises. We took a hands-on approach to learning about how the equipment was used - I even got to do some of the exercises on the HEM myself, and to watch the DEXA scans be done on a few people that came in. This phase of my internship was important because it familiarized me with the study and with the resources I would need to use for future research.

Once we finished our initial research, we decided on a method to go with to model the experiment. First, we would recreate the HEM in a computer-aided design (CAD) program called Solidworks. I had experience with the software already, so it was just a matter of measuring the dimensions of the machine, looking over the blueprints, and drawing it all up. Vicki and I were able to complete it in about a week. Our next step was to try to somehow integrate our CAD drawing with another model of the human musculoskeletal system.

Part of our original idea was to actually use CAD to create the musculoskeletal model, but after exhaustive efforts, we found out that CAD really didn't handle the kind of applications we had hoped it would. So, we looked to some other alternatives that we had come up with before. To get a rough idea of what kind of calculations we could expect once we found a good biomechanical simulation program, we did some “pen and paper” analyses of the exercises. We used the methods I had learned in my biomechanics class, where we drew force-body diagrams of

the exercises, to roughly calculate how much force the major active muscle groups were producing. This was an important phase in my internship because it allowed me to directly apply some of the techniques that I had learned in school.

Once we got our estimates for the gluteal muscle forces, the search was on for simulation software. The first program that we tried was called LifeMOD, which seemed to have a strong potential. One interesting feature it had was that it could actually import data from a “motion capture system” in the nearby Exercise Physiology Lab. This motion capture system was created by placing special markers on important locations on a person’s body, and then filming that person from various angles simultaneously while they performed any exercise(s). We actually used the motion capture system for our one-legged squat. LifeMOD was supposed to be able to perform what is known as “reverse dynamics” analysis on the motion capture data, where the motion of the person is used to calculate the forces acting on his body (e.g. muscle forces). Unfortunately, we found out that a crucial file was missing from our copy of LifeMOD, so that we could no longer use it for our simulation. However, it was interesting to learn about how to use motion capture equipment, and it wouldn’t be long before we found a new program.

The second program we used was called AnyBody. While this program was still in its developmental stage, it had enough to give us a fairly good simulation of our exercises. With our remaining time at NASA, we learned how to use AnyBody to recreate our exercises and get the calculations we needed. AnyBody could not take in motion capture data, so we built rough estimates of how the subjects moved into our models. Similarly to LifeMOD, AnyBody used the information we gave it about the forces on the body and the motion of the body to perform reverse dynamic analysis, and calculate the muscle forces. After comparing our calculations from AnyBody to the “pen and paper” calculations, along with some calculations from similar studies, we decided that our results were accurate enough. We finally had the forces in the gluteal muscles during the hip abduction and one-legged squat, hence completing the main objective of our project.

Conclusions

The obstacles I faced at NASA were quite different than what I had expected. One thing I felt was missing was a mentor with a thorough background in engineering, who could provide concise answers about how to approach the problem and give feedback on our calculations. It often happened that we had to spend a lot of time figuring out what tools to use to solve our problems, and we had to struggle to find out whether our results were valid enough to draw any conclusions. While this may have taught us how to be more resourceful, it often left us without a sense of direction. We also spent considerable amounts of time trying to organize our own project so that we had some tangible results by the end of it. It might have been better if we had specific objectives waiting for us at the start of our internship, so that we knew what we were reaching towards. The aspects of it that I appreciated the most were: the people that I worked with and the diversity of tasks I was given. The entire bone team helped in some way, but especially: Dr. Linda Shackelford, John DeWitt, Scott A. Smith, Mary Jane Maddocks, and of course Vicki Maul. Each of them contributed in their own way: Dr. Shackelford was especially helpful in explaining the study in a clinical sense; Scott helped with a lot of software issues; John helped explain biomechanical principles, the motion capture device, and biomechanics programs; Mary Jane helped with retrieving information and explaining the equipment; Vicki helped in nearly every step of the process. Without their assistance, I would not have gotten nearly as much done. I also got to do everything from design a CAD model, to weight lifting, to biomedical research, to collaborating with people in Denmark for software advice. The things that could have used some improvement were: direct mentorship, and organization. In the end, I realized that I while I would not mind continuing on a more research-oriented path in biomedical engineering, I would really like to experience what the design side of BME is like – especially at NASA.

Running head: NSBRI SUMMER INTERNSHIP 2003

NSBRI Summer Internship 2003: Research at the Johnson Space Center in Houston,
Texas for the Human Environmental Factors Office

Leah Zidon
Truman State University

September 10, 2003

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Experiences I encountered this summer at the Johnson Space Center were exciting, interesting, and educational. Although I have done plenty of research in the past, I found this to be a unique experience working in a professional setting. Many aspects of my education as an exercise science major were useful, although I learned more at JSC than I felt I had brought into the project. I gained experience dealing with frustrations that came with the research and figured out good ways to find solutions to my problems independently.

Much of the first week was spent simply interviewing other employees and learning about their research. Dr. Brian Peacock, my mentor, was still finishing his vacation in England when I first arrived, so some of the other employees gave me journal articles to read and information to learn during this time. I found that there was a lot to gain learning about space travel in general and the processes that go into every mission. The initial few weeks enhanced my education as an exercise science student because I studied functional anatomy, biomechanics, anthropometry, human motion and posture in general.

When my mentor returned to Houston, we discussed my responsibilities. He told me that I should somehow come up with a type of a language or tool to describe posture and motion in microgravitational conditions. This tool should be easy, sufficiently accurate and useful. Other employees in my department should be able to use my tool for quick analysis of posture and motion in space. I found that this was not nearly as easy as it sounded, and I learned new ways to find better solutions to some of my problems. My mentor did not approve of some of my first attempts, and it made me feel more frustrated than ever. I learned how to deal with these frustrations and move on with the research. I

also dealt with many different types of challenges that arose and learned a great deal about working with others in this type of setting.

Eventually I asked my mentor what he wanted more specifically. He told me to research a type of language for dancers, called labanotation, and try to develop something that was similar to that model. The problem that I encountered with this language was that it was difficult to understand; I needed to create something that any NASA employee could learn how to use within minutes. My first solution looked like a stick with dot-to-dot numbers on it, but I felt as though I had found a breakthrough idea that would change the world. My “tool” used easy abbreviations of motion description to describe microgravitational motion. I used three different planes of view so the two-dimensional image that was captured from pictures or video could be transformed into a three-dimensional figure. Orientation of the hands, feet and head were included, as well as location of the human figure in the spacecraft.

Although my first attempt was a good start, there were plenty of problems. Eventually, it would need to be transformed into a computer program, and my first attempt would not have been easily transferable. I decided to switch my focus to a type of graphical method. Dr. Peacock told me that he would help me find a way to transform my thoughts for the computer program, Microsoft Excel. I decided to name the “tool” Microgravity Posture, Movement, and Force Analysis (MPMF). Testing this tool using photos and video proved to be an interesting experience. I encountered several problems with the MPMF, and it slowly transformed over a period of a few weeks.

As motion was introduced as a period of ten frames of pictures, the graph became a complicated mess. I asked different experts for help. Jim Maida, a mentor at NASA,

gave me some new suggestions. I made the graphs more similar to real-life imitations. Eventually my idea progressed, and I created my product for the summer, the MAP-M (Microgravitational Analysis of Posture and Movement).

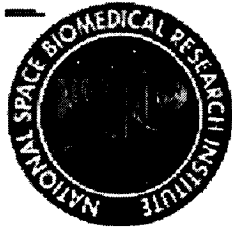
The new “product” now needed different subjects to test simplicity, accuracy, and usefulness. I found 10 different people, including some individuals outside my division, to test my tool. I gave each subject a packet complete with instructions, a sample completed MAP-M graph, frames to be analyzed, and 10 sheets of blank MAP-M graphs to be filled out manually. It took me longer to figure the results than I had originally anticipated, and I did not have time to work the statistics before I left. Another intern will follow up with statistical results on my project in the future. It will eventually be made into an easy-to-use program with the use of a touch-screen tablet.

This summer has been one of the best experiences of my life. I enjoyed my time at the Johnson Space Center while I learned several different applications of my major and other interesting fields. I would suggest this internship opportunity to any student in the future.

I plan to graduate this upcoming May, 2004, with a degree in exercise science. Another internship at the Johnson Space Center is in my future plans, possibly through NSBRI again. I would also like to look into a future with the graduate co-op program for NASA. Within a year, I will go back to school in pursuit of a doctoral degree in an exercise science related field, possibly exercise physiology or biomechanics. My first choice for a doctoral program is at the University of Oregon in their Department of Exercise and Movement Science, which would combine both exercise physiology and

biomechanics. Thank you for supporting me through an educational and enjoyable experience.

Appendix N



National Space Biomedical Research Institute

Commercializing NSBRI-specific Intellectual Property:
Essential Ingredients, Processes, and Recipes for Success.

19 February 2003

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Executive Summary

This report contains a high-level analysis and recommendations resulting from our 17 January 2003 visit to the National Space Biomedical Research Institute in Houston. The purpose of our visit was to gather information and make first order recommendations to increase the NSBRI's effectiveness at commercializing intellectual property (IP) arising from NSBRI supported research and development. The meeting was jointly hosted by the NSBRI Director and the Chairman of the NSBRI Industry Forum. During the meeting, we learned that NSBRI has three core missions: (1) developing counter measures, in partnership with NASA, to reduce the adverse biomedical effects of space travel on humans, (2) education, including mobilizing and disseminating information and knowledge gained through these discoveries to the interested public, and (3) "commercializing" the IP created. NSBRI management stated that to date approximately 85% of the organization's efforts has been focused on the first goal of discovering counter measures. NSBRI is now ready to begin sustained efforts toward achieving the IP commercialization goal.

During our visit we jointly defined long term success in IP commercialization as sustainably and profitably designing, manufacturing, and selling products or services based on NSBRI-funded scientific discoveries. This can be accomplished, in part, through direct licensing of an invention or technology to existing companies or through the creation of new companies to commercialize the invention/technology.

This report first establishes the Requisite Ingredients for successful commercialization of IP: (1) Technology: creating a ground breaking, one-of-a-kind technology, (2) People: being able to acquire top talent with previous start-up experience in the relevant domain, (3) Market: having an identified, medium to large, addressable and growing target market, (4) Business Model: having a capital-efficient business model that produces sustainable, positive cash flow, and (5) IP Control: owning or being able to acquire and assign legal control of the IP.

Next, we suggest three different methods, or "recipes" to combine the Requisite Ingredients: (1) Bifurcated Organization: creating a separate entity to focus on commercialization while retaining the existing organization to focus on the core mission of counter measure discovery, (2) Targeted Communication: specific targeting of communication to people or organizations which are most likely to ascribe commercial value to specific discoveries and science being pursued by NSBRI, and (3) Increased Awareness: generally increasing external awareness of the NSBRI as an organization through external marketing and public relations efforts. These methods are presented as distinct choices and are listed in order of decreasing complexity and decreasing resource consumption. However, the reader will see that these recipes are linked in a quasi-serial fashion with each less resource-intensive option creating the infrastructure for the next, more complex, more comprehensive, more resource-hungry solution.

Given our current understanding of the NSBRI, we recommend near term efforts be focused on acquiring the assets and implementing the processes which will enable the Targeted Communication approach. We believe this approach represents the best balance of risk and reward for the required investment while providing many of the needed components for more extensive approaches in the future.

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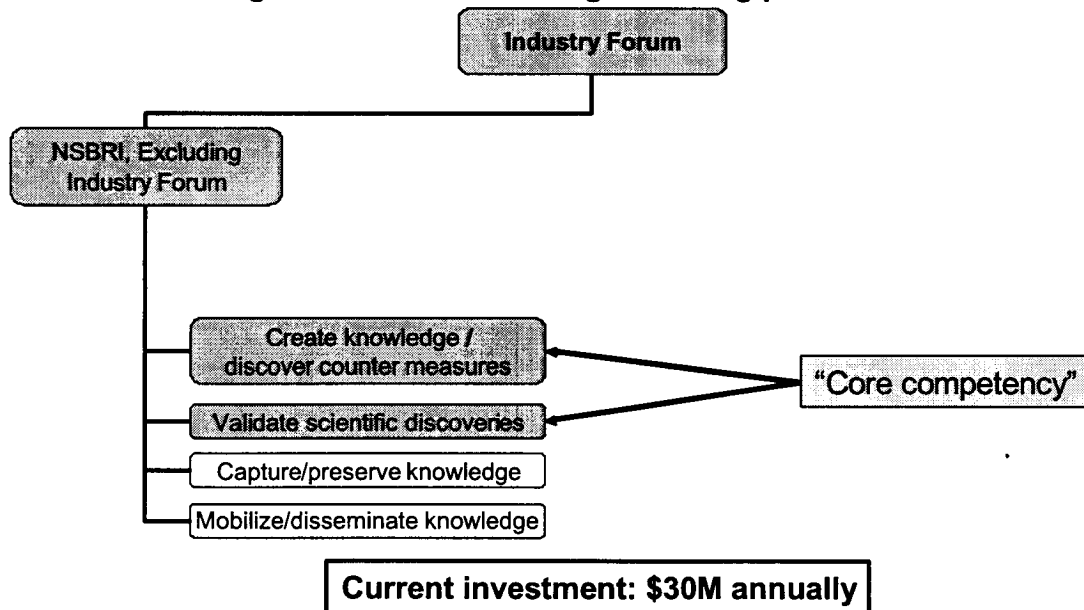
Part 1: Necessary Ingredients for IP Commercialization: Processes and People

As with any complex process, commercializing intellectual property requires the proper ingredients to exist in the right amounts before one can hope to achieve the desired outcome. Once these ingredients exist in the proper amounts, they must be assembled in the correct manner to be successful. In the process of commercializing intellectual property, the necessary ingredients include:

- Technology: Ground breaking, one-of-a-kind technology
- People: Top talent with previous start-up experience in the relative domain
- Market: An identified, medium to large, growing, addressable target market
- Business Model: A capital-efficient model producing sustainable, positive cash flow
- IP Control: The ability to acquire and control IP rights to capture economic value

To gather or create these ingredients, specific processes must be implemented which will produce each ingredient. This list of ingredients should not be considered comprehensive, but should serve as a starting point for a gap analysis of the NSBRI's capabilities. This section investigates individual processes that need to exist and profiles the types of people best suited to execute each process. NSBRI is unquestionably qualified to achieve its main focus which is to discover counter measures (i.e. ground breaking, one-of-a-kind technology).

Figure 1. NSBRI's existing "enabling processes"



In Fig. 1, we list the processes which we identified as existing within the NSBRI framework. We shaded the boxes for the processes that we believe are complete enough at NSBRI to support IP commercialization. The NSBRI's core competency is in the discovery, creation and validation of counter measures. Complementary assets include the ability to capture, preserve,

and to some extent mobilize the information. However, from our initial investigation we do not believe these complimentary assets are yet robust enough at NSBRI to support IP commercialization. To indicate the lack of process robustness, we left two process blocks in Fig. 1 unshaded.

Technology: Ground Breaking, One-Of-A-Kind Technology

NSBRI has assembled what is likely the world’s premier group of scientists and engineers to push the limits of biomedical technology as it relates to extended space travel. The group consists of premier research institutions and scientific thought leaders with an experienced Industry Forum and mission support personnel. The table below articulates the necessary processes and people to create the ingredient – ground breaking technology – from scratch.

The NSBRI’s current configuration enables processes that are necessary, but not sufficient, for the successful commercialization of biomedical intellectual property. Specifically, they make it possible to create new knowledge through discovering counter measures, validating those discoveries, and, to some extent, capturing and mobilizing the knowledge within the group.

TECHNOLOGY	
Enabling Processes	Required People (✓ Present at NSBRI)
<ul style="list-style-type: none"> • Discover counter measures • Validate discoveries • Create knowledge • Capture knowledge • Mobilize knowledge 	<ul style="list-style-type: none"> ✓ Scientific leaders ✓ M.D.s and Ph.D.s ✓ Research universities

People: Top Talent With Previous Start-Up Experience In The Relevant Domain

When commercializing technologies, it is essential that the original inventors of the technology become an active part of the new enterprise’s founding team. Rarely have there been cases where an inventor has been able to hand a “users manual” to another scientist who then goes on to create a successful enterprise. A mechanism should be designed to allow NSBRI sponsored scientists to temporarily leave the organization with their discovery during the early stages of commercialization.

In addition to leveraging internal talent, the NSBRI will need to attract people with a complimentary business skill set gained from hands-on experience with start-up companies. Great entrepreneurs are risk-seekers that will be attracted by the existence of a ground breaking technology and an initial piece of capital to prove out the business. There are several ways to attract this talent, but one tried and proven way to get

PEOPLE WITH COMMERCIAL EXPERIENCE	
Enabling Processes	Required People (✓ Present at NSBRI)
<ul style="list-style-type: none"> • Attract external talent • Provide seed funding • Identify/reallocate NSBRI human resources (R to D) • Identify sources of external funding • Increase external awareness 	<ul style="list-style-type: none"> * Recruiting experts with access to talent pool * Previous “deal experience * Relationships with potential funding sources * Experienced entrepreneurs

the attention of the right people is to have a well-publicized initial success. This is truly a case where success breeds success and the seed could likely be planted by working with a group that has access to and influence among great entrepreneurs.

Market: An Identified, Medium To Large, Growing, Addressable Target Market

Most experienced business people will agree that finding the right market is absolutely crucial to the success of any commercialization initiative. Specifically, the invention should solve a problem that is currently “costing” a specific group of people or companies more than \$300M annually. This market should be experiencing year-over-year growth of 20% or more. Because the NSBRI is driven by biomedical science, it will be most effective to map the intellectual property to the market place and determine what market needs (if any) the discovery satisfies. The good part about this ingredient is that it is likely to exist, so we don’t need to create it from scratch. We just need to know it when we see it.

The table to the right identifies the types of people needed and enabling processes they will create. The time of these required people could easily be leveraged with the time of current MBA students. In top MBA programs, students are well qualified to help with this analysis provided they have relevant pre-MBA experience

MARKET IDENTIFICATION	
Enabling Processes	Required People (✓ Present at NSBRI)
<ul style="list-style-type: none"> • Map Intellectual Property • Identify Market Opportunities 	<ul style="list-style-type: none"> × Strategic IP Experts × Market Research Analysts × Previous biotech knowledge/experience

and access to more experienced “practioner faculty” members. Hiring summer MBA interns for this role would be a very good way to minimize expenses and maximize returns. However, careful candidate selection will be important to insure quality results.

Business Model: A Capital-Efficient Model Producing Sustainable, Positive Cash Flow

Once a ground-breaking technology exists, a growing, addressable market is identified, and a stellar team is in place, the next question becomes, “How do we choose to build this product (or provide this service) and how do we ask our customers to pay us?”

Experienced analysts and consultants are capable of devising and validating business models. However, there are several tried and true business models that are usually recognizable to second year MBA students. At some MBA programs, hands-on courses exist where students assist start-ups with exactly

BUSINESS MODEL	
Enabling Processes	Required People (✓ Present at NSBRI)
<ul style="list-style-type: none"> • Create Business Models • Validate Business Model 	<ul style="list-style-type: none"> × MBA or Experienced Analysts (perhaps Current MBA Students with “parental guidance”)

these types of issues. One such course is the Entrepreneurship Laboratory taught by Ken Morse at the MIT Sloan School of Management.

IP Control: Ability To Acquire IP Control And Assignment Rights

This is one of the more challenging bottlenecks to achieving the goal of commercializing NSBRI’s IP. Sorting out ownership issues is often challenging at a single university which has multiple sources of funding. The problem is even more daunting in the case of NSBRI where discoveries may be made by collaboration among several diverse individuals at different universities using different sources of funding. Highly specialized and skilled individuals with prior licensing experience at large universities or similar experience dealing with these issues at a private law firm will be necessary to sort through these issues. The table to the right articulates the required processes and people to have this “IP commercialization ingredient” in sufficient quantity.

CONTROL OF IP	
Enabling Processes	Required People (✓ Present at NSBRI)
<ul style="list-style-type: none"> • Resolve IP Ownership Issues • Established Guidelines For Resource Contributions 	<ul style="list-style-type: none"> * Previous Licensing Office experience * J.D./MBA

Once general guidelines are in place, less experienced individuals (i.e. less expensive) should be able to execute on the established process within the guidelines.

The ultimate goal is to implement a repeatable process that enables resolution of the entangled control rights for current and future discoveries.

Part 2: Three Recipes For NSBRI IP Commercialization: Bifurcated Organization, Targeted Communication or Increased Awareness

We have identified the necessary ingredients for IP commercialization, as well as the processes and human resources needed to create each ingredient from scratch. As with any complex process, there are an infinite number of “recipes for success.” In this section, we will suggest three such recipes that we believe will increase the chances for successful commercialization of the NSBRI’s IP. Specifically, the three approaches are:

- **Bifurcated Organization**: Bifurcate the existing NSBRI organization, creating a separate organization to focus on commercialization
- **Targeted Communication**: Specifically target and communicate to people or organizations which are most likely to ascribe commercial value to specific discoveries and science being pursued by NSBRI
- **Increased Awareness**: Generally increase external awareness of the NSBRI as an organization through marketing and improved public relations

Creating a Bifurcated Organization is the most comprehensive approach to the problem. Consequently, it is the most expensive, the riskiest, and if successful promises to maximize the NSBRI’s IP commercialization potential. The other two options are successively less costly and

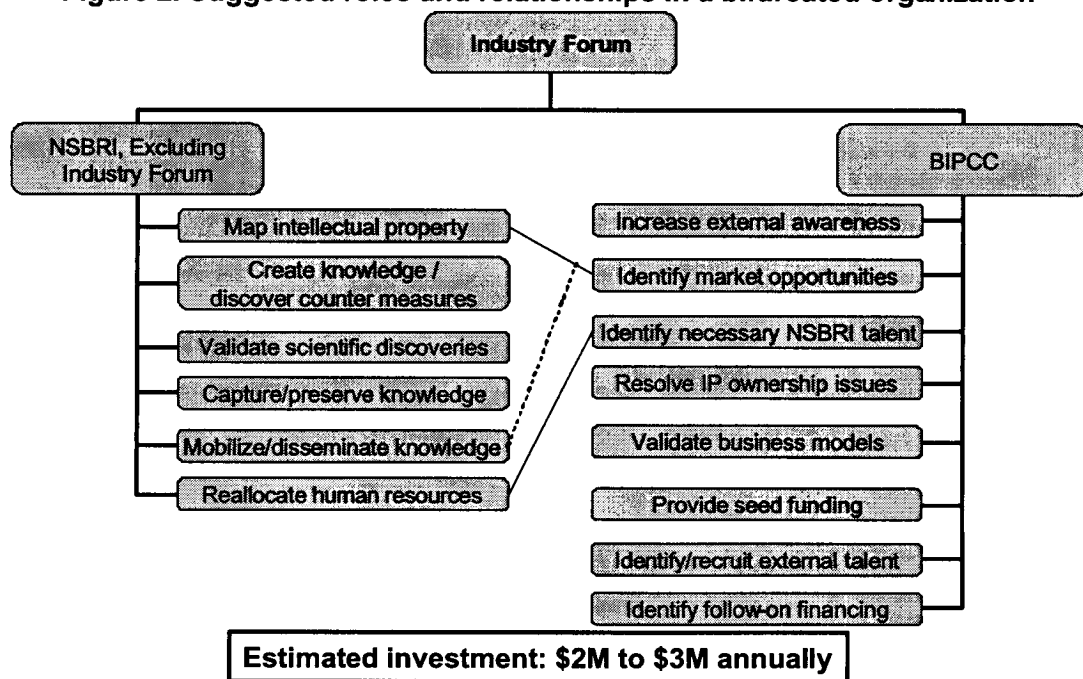
less risky, but also offer less potential return if considered as a static solution. However, the second two options have been designed so that they can serve as building blocks towards a Bifurcated Organization.

Our advice is to pursue Targeted Communication, which also encompasses the initiatives necessary for Increased Awareness, but stops just short of creating two separate entities.

Bifurcated Organization: Create Separate Organizations: Achieves Goals At Great Expense But With No Guarantee

In Fig. 2, we show each of the different “enabling processes” identified in the previous section under the correct entity – the NSBRI, or the commercialization arm that was arbitrarily named the Biomedical Intellectual Property Commercialization Center (BIPCC). The boxes are shaded to indicate that all of these processes, people, and ingredients will exist in the proper amounts, should the financial and organizational commitment be made to pursue a Bifurcated Organization.

Figure 2. Suggested roles and relationships in a bifurcated organization



If NSBRI’s goal is to maximize the organizational potential to commercialize IP at any cost, the required financial resources will be significant and some of the human capital will need to be found outside of NSBRI. It is likely that this effort would have a higher chance of success if two separate entities – with separate budgets, human resources, and incentive structures – were created. The existing NSBRI would remain intact, but have a renewed focus on discovering counter measures and be compensated by establishing and achieving scientific milestones on the path to discovery of identified countermeasures. Consequently, NSBRI publications for the dissemination of information should be a byproduct of the effort and used as an indicator of progress, not a driver.

The second entity, the BIPCC, would be tasked with the dissemination of the information created by the NSBRI and the commercialization thereof. The BIPCC would be comprised mainly of entrepreneurs who have successfully started and built organizations, guiding them from scientific discovery through to cash-flow positive businesses. BIPCC's incentive scheme would need to measure and compensate individuals on milestones and achievements associated with commercializing scientific discoveries. The dissemination of information is a necessary, but not sufficient task to make this process successful.

The establishment of BIPCC as a separate entity is recommended because the skill sets, cultures and reward structures required to achieve successful commercialization are quite different and perhaps even orthogonal to those required to meet NSBRI's core counter measure development goals.

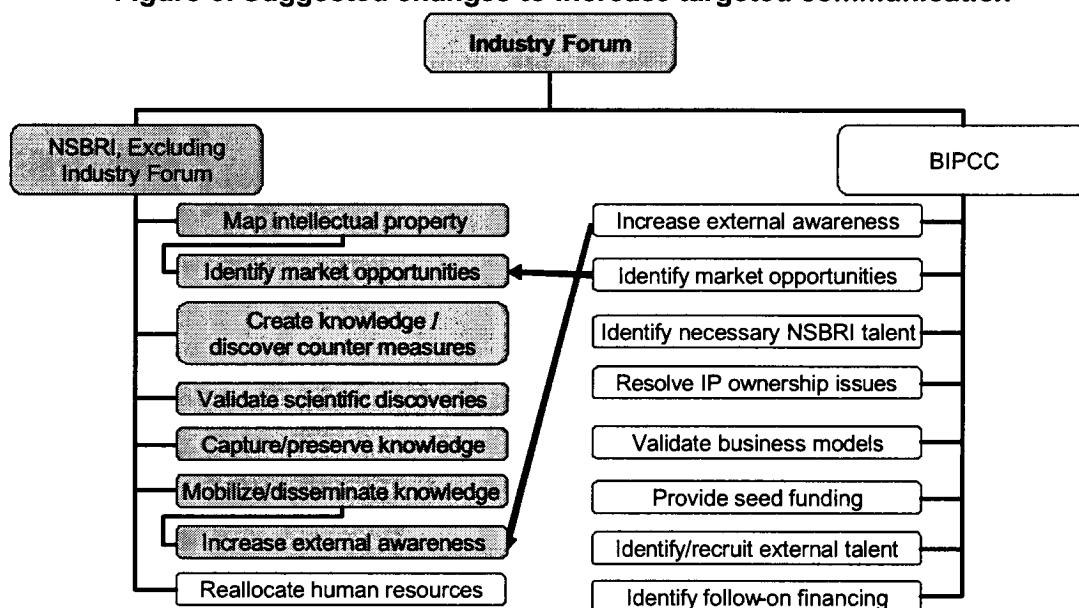
A Bifurcated Organization is the most complete and comprehensive approach to increasing the effectiveness and efficiency of the NSBRI's IP commercialization effort. However, the resources necessary to design, implement, and seed this approach are significant and to our knowledge there is no previously successful government-run model to use as a guide. The stress this type of comprehensive change might place on the existing entity, combined with the uncertainty of the outcome and projected resources needed, is likely to make this an option to be reserved for future consideration. However, with the proper time frame and approach this could be a likely long-term solution.

Targeted Communication: Increase Targeted Education: Enables Progress With Existing Team And Reserves Options

Although the existing NSBRI team's core competency is generally focused on its ability to make scientific discoveries, many of the members are also quite adept at publishing scholarly articles and obtaining patents. The crucial step in commercialization is communicating the specifics of intellectual property to the persons who have needs and interests in the commercial possibilities created by the intellectual property. Many innovations are never commercialized because of the failure of this crucial step.

Subsequent to our January visit, we had a conference call with NSBRI to better understand the existing capabilities of the current IT infrastructure. The infrastructure described to us is impressive – well advanced beyond what we have seen at many universities and research labs. We believe that this IT foundation can be leveraged by using recently available new technologies for IP analysis, knowledge management, and collaborative communication. Using these tools, the NSBRI can increase the awareness and availability of NSBRI information for targeted and interested parties. This should effectively extend NSBRI's current capability and identify intellectual property gaps that an interested party could proactively pursue with NSBRI intellectual property. One very important step accomplished by this approach is that it begins to establish the infrastructure necessary for a full-blown commercialization effort.

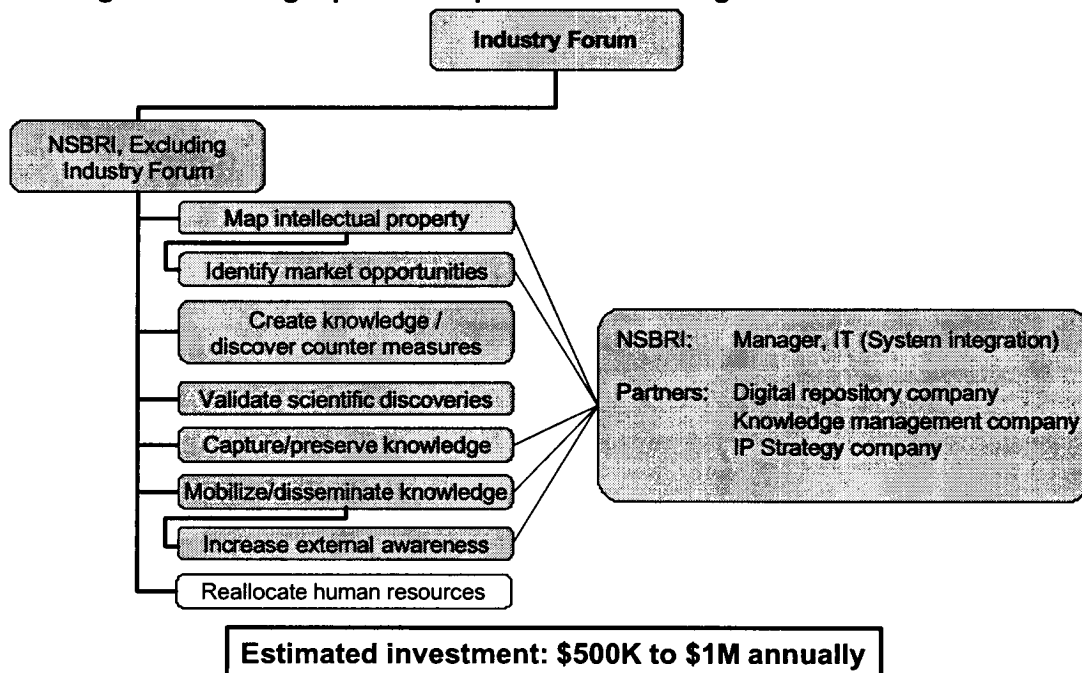
Figure 3. Suggested changes to increase targeted communication



In this intermediate step to bifurcation, some processes would initially fall under the NSBRI's umbrella to be later transferred to the BIPCC at the appropriate time (see Fig.3). Of course, this recipe reserves to management the option not to move forward with bifurcation, should the Targeted Communication approach prove to be sufficient.

In this model, the information technology (IT) infrastructure becomes essential. Consequently, management should consider using an IT solution that has little risk associated with it. To the extent possible, the IT system should be comprised of solutions from established software vendors. Using established vendors insures that software updates and revisions, patches, etc. are likely to be created as long as the company is in business. Also, by using established vendors attrition of individual employees is mitigated as a risk factor. This is not the case with highly customized solutions. However, it is true that significant integration will be necessary because of the special requirements of NSBRI's mission, requiring a highly skilled IT staff as a software integrator and software vendor interface for the NSBRI (see Fig. 4).

Figure 4. Strategic partnerships to increase targeted communication



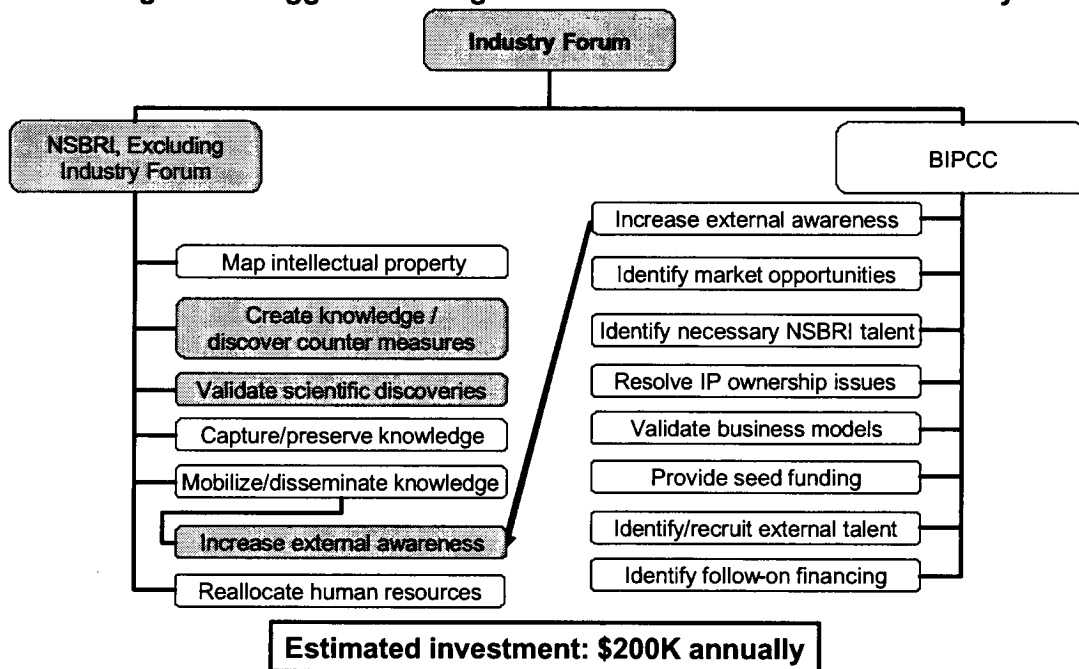
Building out this infrastructure will be beneficial for NSBRI's commercialization efforts and is also likely to be well received by, and beneficial to, NASA based on the input we received during our visit. In this approach, the NSBRI can bring subject experts in on an as-needed basis rather than hiring people outright.

The main advantages of this solution are that it is a measurable move in the right direction, it is achievable, and it reserves the option for a more comprehensive approach.

Increased Awareness: Addresses Awareness At Low Expense But Limited Progress

There is an argument that the only problem the NSBRI is facing is general lack of public awareness of its existence and mission. We have personally noted two examples of this since returning from Houston. First, the portable spectrometer shown to us at the NSBRI was featured in last month's issue of MIT Technology Review with no mention of the NSBRI. Second, we noticed the portable ultrasound monitoring device that was developed by NSBRI investigators is on display at the Boston Museum of Science. Again, there is no mention of the NSBRI's efforts.

Figure 6. Suggested changes to increase external awareness only



If NSBRI management believes the gating item to commercializing its technology may be just a lack of external awareness, or if financial resources are so tightly constrained that small moves are all that is possible, one approach would be to engage a public relations or marketing firm. This could also be an orchestrated move in the right direction. The shaded parts of Fig. 6 depict a comprehensive view of organizational capabilities if this change were implemented and how it fits into the big picture, i.e. Fig. 2. It is recognized that the NSBRI has an installed process to capture, preserve, mobilize, and disseminate knowledge, but again these blocks were left unshaded to indicate our opinion that more resources may be necessary in this area.

Part 3: Conclusions & Recommendations

Given the current stage of the NSBRI as an organization, we recommend that NSBRI's near term efforts be focused on acquiring the necessary assets and establishing the processes necessary to implement the Targeted Communication approach. We believe this approach offers the best balance of progress, associated risk, and potential reward over the next several years. This option allows for less expensive and controlled changes, while addressing what we believe is the core challenge facing the NSBRI for IP commercialization.

We also recognize there may be a desire to achieve a short-term win to create and build momentum, so a hands-on approach attempting to commercialize a few of the most promising technologies could be started simultaneously with the Targeted Communication initiative. These case studies would help in many ways, including identifying additional problems that will be encountered during the commercialization process. The knowledge from lessons learned with these test cases can be incorporated into the overall solution.

If desired we are prepared to assist NSBRI in this regard by preparing a resource and action plan to implement the Targeted Communication approach.