Bion-M2 PI 1-pagers

Project Manager: Diana Ly
Mission Scientist: Dennis Leveson-Gower, Ph.D.

Single cell analysis of bone marrow progenitor and differentiated progeny populations in response to long-duration spaceflight

(NASA Proposal #18-BM2-Ph2-0019)

PI Flight Investigation

PI: Elizabeth Blaber, Ph.D., Rensselaer Polytechnic Institute

Previous spaceflight research has shown a dysregulation in both embryonic and somatic stem cells proliferation, differentiation, and regeneration capacity in microgravity conditions, which may be the cause of the widespread tissue degeneration observed in space. However, the molecular mechanisms activated by spaceflight conditions in stem cell populations are not completely understood. This is in part because the complex and heterogeneous nature of the bone marrow compartment prevents changes in whole tissue genomic patterns from being attributed to specific sub-populations. In this investigation, we seek to understand and characterize the microgravity-induced molecular genomic changes that occur at the single cell level enabling the identification and characterization of individual cell and molecular responses to the spaceflight environment and provide insight into the molecular basis for the observed shifts in cellular phenotypes.

Objective:

- Test the hypothesis that gene expression changes induced in bone marrow cells by spaceflight are not gradual and continuous, but instead are the result of quantum gene expression changes induced in bone marrow cells, and therefore, the identification of these changes in quantum state will identify underlying regulatory mechanisms and characterize the effects of spaceflight on bone marrow progenitor cells and their differentiated progeny.
  - SPECIFIC AIM 1: Determine the transcriptional changes that occur at the single cell level in bone marrow stem cells and differentiated progeny populations.
  - SPECIFIC AIM 2: Compare the effects of microgravity and re-adaptation/recovery on hematopoietic and mesenchymal stem cell lineages in weight bearing versus non-weight bearing bones.
  - SPECIFIC AIM 3: Determine if spaceflight stress cause alteration to hematopoietic stem cell (HSC) niche populations in the bone marrow compartment eliciting HSC and differentiated progeny to redistribute to the spleen and liver following long-duration spaceflight exposure.

Experimental Approach – Following recovery from the Bion-M2 capsule, mice will be euthanized and dissected at 4 post-landing timepoints; 1 hour, 12 hour, 5 days, and 15 days. The following tissues will be collected: blood, liver, spleen, and bone marrow and skeletal tissue from pelvis, femur, tibia, humerus, rib, and cranium. To study the effects of microgravity exposure and re-adaptation on various “load centers” of the mouse, bone marrow will be extracted from 3 different sites; *pelvis, femur and/or tibia* are normally loaded at 1G but unloaded in microgravity, *humerus, ribs, thoracic vertebrate* are normally unloaded at both 1G and in microgravity, and *calvarial bones* are normally unloaded at 1G but loaded in microgravity. Bone marrow cells from each of these sites will be extracted by flushing with phosphate buffered saline and frozen or fixed in RNA preservative for single-cell transcriptome sequencing.

Relevance/Impact – This investigation will elucidate the cellular and molecular mechanisms associated with spaceflight-induced changes to stem cell-based regeneration and repair with unprecedented single cell resolution. Results will provide insight into the reversibility of these effects with reloading at 1G. This study addresses Space Biology Research Plan's questions SBP CMB-1, CMB-3, CMB-4, CMB-5, and CMB-8, and SB AN-2 and AN-3, the Decadal Surveys calls AH3, AH14, and CC8, and addresses Science Emphasis 1: *Physiology of the mouse cardiovascular system, fluid regulation that might be expected to influence intracranial pressure, musculoskeletal system, endocrine function, the microbiome, and re-adaptation.*

Exploration benefit – Development of countermeasures for the detrimental effects of microgravity and radiation exposure on stem cell-regenerative health in tissues, which are applicable to the prevention of widespread tissue degeneration and to facilitate long-term spaceflight.

Earth benefit – Dysregulation of basic stem cell functions results in impaired abilities to proliferate, differentiate, and repair damaged tissues, similar to the regenerative decline that occurs during aging. The characterization of regulatory mechanisms and effects on bone progenitor cells may elucidate the mechanisms of osteoporosis.

The Effects of Spaceflight and Reloading on Skeletal Muscle and Bone
(NASA Proposal #18-BM2-Ph2-0021)

PI Flight Investigation

PI: Mary Bouxsein, Ph.D., Beth Israel Deaconess Medical Center

Musculoskeletal response and the effects of reloading on skeletal muscle or bone during long-duration spaceflight remains ill-defined. Our overall objectives are to assess musculoskeletal response and readaptation to the Earth's gravity following long-term spaceflight and to advance knowledge regarding the underlying physiological and molecular mechanisms contributing to bone and muscle recovery and their interactions. We aim to assess functional, structural, and molecular alterations in skeletal muscle and bone during reloading and identify candidate mechanisms that will lead to better recovery strategies following long-term spaceflight and disuse-induced osteoporosis on Earth.

Objective:

- Test the hypothesis that inflammatory and autophagic responses precede the canonical Wnt and mTOR signaling pathways to promote bone and skeletal muscle regeneration during reloading and identify novel unexplored mechanisms that may lead to new therapeutic interventions.
  - SPECIFIC AIM 1: Determine effect of combined exposure to increased space radiation and microgravity on structural and functional alternations in skeletal muscle and bone.
  - SPECIFIC AIM 2: Determine structural and functional changes in skeletal muscle and bone during readaptation to Earth's gravity following exposure to space radiation and microgravity.
  - SPECIFIC AIM 3: Determine gene expression profiles for bone and muscle following exposure to space radiation and microgravity, and during subsequent reloading on Earth.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice will be euthanized and dissected at 4 post-landing timepoints; 1-3 hours, 15-17 hours, 5 days, and 15 days. The following tissues will be collected and frozen or fixed: right tibia, right and left femur, right and left soleus. Muscle and bone structure will be assessed by histology, histomorphometry, and micro-computed tomography (microCT). Bone function will be assessed by micro-finite element analysis, and fracture toughness testing. Protein and gene expressions will be assessed by immunohistochemistry, Western blot, direct mRNA transcript analyses, and whole transcriptome sequencing.

Relevance/Impact – This investigation will examine the possible muscle-bone crosstalk and associated molecular mechanisms of reloading. With increasing evidence of crosstalk between muscle and bone, systematic interrogations of muscle and bone alterations may allow development of pharmacologic, nutritional or biophysical interventions to promote faster and safer musculoskeletal recovery during reloading. This proposal addresses Space Biology Research Plan's questions SBP CMB-1, CMB-2, CMB-3 and SB AN-3, and addresses National Research Council's AH3 and AH5 on bone and connective tissue.

Exploration benefit – Despite advances in exercise and pharmacologic countermeasures designed to mitigate musculoskeletal deterioration, the protection is incomplete and response to interventions variable. Countermeasures to the deficiencies in musculoskeletal structure, function and neuromotor performance are essential for short- and long-term missions in order to mitigate musculoskeletal rehabilitation.

Earth benefit – Development of countermeasures and rehabilitation strategies to mitigate disuse-induced osteoporosis and recovery from immobilization, as well as to improve function and reduce secondary injuries after astronauts' return to Earth.

High Altitude Spaceflight on the Bion-M2: Effects on Arterial and Venous Vessels

(NASA Proposal #18-BM2-Ph2-0012)

PI Flight Investigation

PI: Michael Delp, Ph.D., Florida State University

Emerging research demonstrates that low-dose environmental and medical radiation exposure increases the risk of mortality due to ischemic coronary artery (heart attack) and cerebrovascular (stroke) disease. Despite the potential for low-dose radiation exposure during spaceflight to have substantial adverse effects on the cardiovascular system, little research has been conducted to investigate this phenomenon. This is particularly urgent as groups of nations and corporate entities are actively planning manned missions back into deep space (i.e., the Moon and Mars), where exposure to galactic cosmic radiation is pervasive. Results from this investigation will lead to a greater understanding of the cardiovascular health risks for deep space travel and address the impacts of spaceflight and radiation on coronary, cerebral, ophthalmic, and jugular vascular function, mechanics, and structure.

Objective:

- Test the hypothesis that spaceflight will impair endothelium-dependent vasodilation in the coronary, cerebral and ophthalmic arteries and internal jugular veins, and diminish coronary blood flow velocity compared to that in age-matched ground control mice. Additionally, it is hypothesized that changes in expression of eNOS will be lower in cerebral arteries during the 5 and 15-day recovery period and that the pro-inflammatory cytokines TNFα and IFNγ will be elevated, while circulating biomarkers of inflammatory and oxidative stress will remain elevated in mice flown aboard Bion-M2 and will remain elevated throughout recovery.
  - SPECIFIC AIM 1: Determine whether a weightless and high space radiation environment on the Bion-M2 satellite and up to 15 days of recovery on Earth alters coronary, cerebral and ophthalmic artery and internal jugular vein vasomotor responses, mechanics and structure, along with other indices of coronary vascular function.
  - SPECIFIC AIM 2: Determine the effects of high altitude spaceflight on the Bion-M2 satellite and up to 15 days of recovery on key markers of nitric oxide signaling, oxidative stress and inflammation in arteries, veins and the blood.

Experimental Approach – Studies will be performed at 3 post-landing timepoints; 14-17 hours, 5 days, and 15 days, to determine cardiac function, coronary blood flow velocity, and aortic pulse wave velocity prior to harvesting tissues for *in vitro* experimentation. Coronary, cerebral, ophthalmic arteries and internal jugular veins will be isolated. For determination of cardiac and coronary function and aortic pulse wave velocity *in vivo*, echocardiography and left coronary artery blood velocity (LCABV) will be utilized. Anatomic and functional cardiac measurements will be made on anesthetized mice. Vascular tissue and blood will be collected to examine key signaling molecules for endothelium-dependent vasodilation, oxidative stress and inflammation, as well as proteomic profiles and pathways. Tissue will be frozen for western blot and quantitative proteomic analyses.

Relevance/Impact – The effects of a low-dose space radiation environment on the cardiovascular system and how space radiation may interact with a weightless environment (simulated or actual) to affect the heart and vasculature are not well defined. This investigation will elucidate the impact of a higher-radiation microgravity environment on the cardiovascular system for long-term missions. This proposal addresses Space Biology Research Plan's questions SBP CMB-1, CMB-3 and SB AN-1, AN-2, AN-3, and Appendix C with emphasis on physiology of the mouse cardiovascular system and the effects of radiation on organism health.

Exploration benefit – Functional and structural alterations in the arteries and veins of the head, eyes and neck could be important contributors to the development of Spaceflight Associated Neuroocular Syndrome (SANS). This investigation will aid in the development of countermeasures for SANS, a visual impairment suffered by up to 60% of astronauts flying on long-duration missions.

Earth benefit – An imbalance of reactive oxygen species (ROS) leads to a number of pathophysiological conditions and impairs function in both arteries and veins, particularly cardiovascular homeostasis. The data from this investigation may be translated to common diseases in humans on Earth, which may lead to novel methods for cardiovascular disease treatment.

The impact of the prolonged LEO on male reproductive health and fertility in mice on the Bion-M2 mission

(NASA Proposal #18-BM2-Ph2-0022)

PI Flight Investigation

PI: Lesya Holets, Ph.D., University of Kansas Medical Center Research Institute

Bion-M1 results showed significant declines in testicular integrity, degeneration and disorganization of spermatogenic cells, appearance of gaps in the epithelium, increased number of residual bodies in the lumen, premature release of spermatids, abnormal Leydig cell morphology, and significant changes in expression of important markers of spermatogenesis and steroidogenesis. Bion-M2 mission gives a unique opportunity to confirm and expand the significant changes in testis histopathology, sperm production and storage, and immune and endocrine regulatory dysfunction during long-term spaceflight. This investigation will determine the effects of spaceflight on spermatogenic cell apoptosis and sperm DNA damage followed by mating trials and/or IVF (in vitro fertilization) to determine actual fertility of the flown animals compared to ground controls.

Objective:

- Test the hypothesis that exposure to microgravity for 30-45 days causes disruption of spermatogenesis and steroidogenesis in adult mice leading to infertility.
  - SPECIFIC AIM 1: Identify specific histopathology and dysfunction in spermatogenesis and testis function in mice exposed to combined space radiation in LEO and microgravity for 30-45 days (as determined by mission duration) and compare to ground-based controls.
  - SPECIFIC AIM 2: Identify specific epididymal histopathology and functions of sperm transport and storage in mice exposed to both, space radiation in LEO and microgravity for 30-45 days and compare to ground-based controls.
  - SPECIFIC AIM 3: Quantify changes in sperm motility and morphology (including sperm viability and sperm DNA damage assay) in cauda epididymal sperm from male mice exposed to both, space radiation in LEO and microgravity for 30-45 days and compare to ground-based controls.
  - SPECIFC AIM 4: Determine genomic molecular networks of gene expression, and dysregulation in gene transcription of important markers of spermatogenic regulation, inflammatory and steroid production, identify altered metabolic pathways in mice exposed to combined space radiation in LEO and microgravity for 30-45 days and compare to ground-based controls.
  - SPECIFIC AIM 5: Determine male reproductive health after post flight recovery by assessment of testis morphology, sperm motility and sperm DNA damage, and ability to insemination (IVF and mating trial) in 15 days post return from the orbit.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice (60 days or older at launch) will be euthanized and dissected at 2 post-landing timepoints; 1-3 hours and 15 days. The following tissues will be collected, photographed, and frozen, preserved in RNAlater or fixed in Bouin's: testis, epididymis, and sperm. Quantitative/qualitative histopathologic, morphologic, sperm DNA damage, next-gen RNA sequencing, and mating fertility analyses will be performed, in addition to *in vitro* fertilization trials.

Relevance/Impact – Bion-M2 will be much closer to the Van Allen radiation belt than previous live animal flight experiments. In this regard, the testes and ovaries are the most sensitive organs to radiation damage at either single or fractional doses and much more sensitive than bone, brain, heart and muscle. This investigation will determine the influence of prolonged simulated microgravity on male reproduction and fertility and on secretion of steroid hormones involved in regulation of physiological functions. This study addresses Space Biology Science Plan Goal for Program Element #5 Developmental, Reproductive and Evolutionary Biology and aligns with Objectives 5.a., 5.b., and 5.c..

Exploration benefit – Long-term spaceflight may have a negative impact on male reproductive health, affecting spermatogenesis, testosterone levels, and health of offspring. This investigation will elucidate the critical biomarkers and pathways that explain the molecular, cellular, and hormonal mechanisms underlying changes in male fertility influenced by spaceflight.

Earth benefit – The data from this investigation will translate to characterization of Earth-based infertility, slow embryo development, and implantation/birth defects.

Biomarkers Associated with Spaceflight-induced Cardiac Dysfunction
(NASA Proposal #18-BM2-Ph2-0008)

PI Flight Investigation

PI: Peter Lee, M.D., Ph.D., MPH, Ohio State University

There are increasing concerns that space radiation will lead to accelerated coronary atherosclerosis and other cardiac problems. Non-invasive biomarkers of cardiac dysfunction (microRNAs)
have the potential to serve as a powerful tool to predict, diagnose, and monitor cardiovascular pathologies in astronauts during and after extended spaceflight. This investigation aims to assess the effect of extended spaceflight in murine hearts through the study of heart function and electrocardiogram (EKG). These findings will be compared to panels of miRNAs that are known to be associated with cardiac pathologies based on previous radiation-associated studies.

Objective:

- To test the hypothesis that extended microgravity and space radiation exposure causes measurable changes in cardiac function, morphology, and rhythm disturbances that correlate with changes in cardiovascular biomarkers.
  - SPECIFIC AIM 1: Determine the effect of extended microgravity and radiation exposure on murine cardiac function.
  - SPECIFIC AIM 2: Determine the effect of extended microgravity and space radiation exposure on murine cardiac rhythms.
  - SPECIFIC AIM 3: Identify potential cardiovascular biomarkers in response to extended microgravity and space radiation exposure in mice.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice (5-6 months old at launch) will undergo anesthesia for non-invasive and non-terminal studies to include transthoracic echocardiography and EKG at 4 post-landing timepoints; 1 hour, 12 hour, 5 days, and 15 days. Post-euthanasia, the following tissues will be collected and preserved: Blood (serum and whole blood) and a portion of left ventricular heart tissue. Transthoracic echocardiographic and EKG exams, echo image analysis, whole blood analysis for miRNA alterations associated to immune functions, and miRNA analysis in serum and heart tissue will be performed.

Relevance/Impact – Despite extensive human studies on astronaut cardiac function, very little is known about the combined effects of microgravity and space radiation. This study will validate the use of these biomarkers in a murine model with exposure to extended periods of microgravity and significant degree of space radiation. This investigation aligns with Space Biology Science Plan Goals; 1) Physiology of the mouse cardiovascular system, fluid regulation that might be expected to influence intracranial pressure, musculoskeletal system, endocrine function, immune function, the microbiome, and re-adaptation 2) Effects of radiation on organism health, biological function, and genetics or other systems biology (omics) parameters, and addresses DEGEN 1, 2, 3, 7 and CV1, 7, and 8.

Exploration benefit – Identifying serum biomarkers that correlate with spaceflight-induced cardiovascular pathologies may have a significant impact on our ability to predict, diagnose, and monitor cardiovascular issues in astronauts during and after extended spaceflight. miRNAs are ideal molecules to be used as such biomarkers as they can be easily analyzed, have unique organ-specific signatures, and may be identifiable at the earliest stages of disease.

Earth benefit – By identifying novel biomarkers that provide readouts of events that are associated with or lead to pathological progression of cardiac diseases and inflammation, novel therapies may be developed for early detection and monitoring of cardiac pathologies.

Bion-M2 Spaceflight-induced Effects on Neurovascular Remodeling and Blood-retina Barrier Function: Role of Oxidative Stress

(NASA Proposal #18-BM2-Ph2-0006)

PI Flight Investigation

PI: Xiao Wen Mao, M.D., Ph.D., Loma Linda University

More than 50% of the astronauts returning from space shuttle missions or International Space Station (ISS) have reported a subjective change in their visual acuity. These visual disturbances
have been hypothesized to be related to increased intraocular (IOP) or intracranial pressure (ICP). However, the underlying mechanisms and pathological manifestations of increased IOP or ICP during spaceflight are currently unknown. The objective of this proposed project is to characterize the effect of Bion-M2 mission on retinal vascular remodeling and visual function. Furthermore, the molecular and cellular mechanisms involving oxidative stress-induced vascular response and impaired blood-retina-barrier (BRB) and blood-brain barrier (BBB) integrity will be investigated.

Objective:

- Test the hypothesis that spaceflight and radiation-induced oxidative stress is responsible for maladaptive vascular remodeling, chronic inflammation and BRB disturbance, thus placing the retina at risk for visual impairment and neurodegeneration.
  - SPECIFIC AIM 1: Define the relationships between spaceflight-induced oxidative stress in reactive oxygen species (ROS) expression, retinal vascular remodeling, and retina function following Bion-M2 spaceflight mission.
  - SPECIFIC AIM 2: Determine whether spaceflight condition alters the blood-retinal barrier integrity and function.
  - SPECIFIC AIM 3: Identify and characterize transcriptional profiles that are specifically associated with oxidative stress-induced neurovascular and tissue response to prolonged exposure to space environment.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice will undergo non-invasive and non-terminal IOP tonometry and electroretinogram (ERG) for retinal function assessment at 4 post-landing timepoints; 1 hour, 12 hour, 5 days, and 15 days. Post-euthanasia, the following tissues will be collected and fixed or frozen: brain and eyes. MRI-diffusion tensor imaging for fixed eye, immunobiomarkers for fixed ocular sections, gene and protein expression profiles for oxidative stress and extracellular matrix measurements by gene array, western blot assays, and immunostaining will be performed.

Relevance/Impact – Various factors have been suggested to account for increased IOP or ICP during spaceflight. Modeling studies have shown that a compromise in the integrity of the vascular barrier function would serve to elevate ICP. Although few studies have examined retinal vasculature and BRB integrity/function following prolonged exposure to spaceflight, evidence suggests the need to investigate the role of oxidative stress in neurovascular hyperpermeability and barrier dysfunction. This study addresses the Decadal Surveys calls P2, AH3, AH5, AH8, and CC8, CC10.

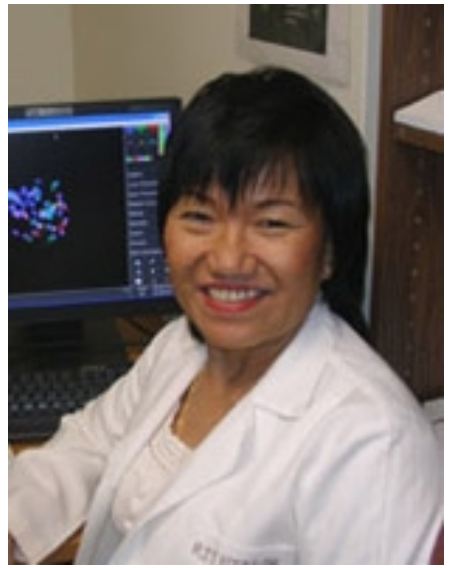
Exploration benefit – Integrative, quantitative and multidiscipline activities with advanced imaging techniques will provide insight into the molecular mechanisms and pathways of spaceflight-induced oxidative damage on retinal vascular structure and BRB integrity. Understanding how factors and environmental insults impact on vasculature and tissue remodeling and function will help focus the approach towards more effective countermeasures during human spaceflight and planetary exploration.

Earth benefit – BRB disruption induced by oxidative stress and other factors are important causes of irreversible blindness in many retinal diseases, including diabetic retinopathy and macular degeneration. Findings from this study may lead to new efficacious therapies that can prevent, reverse or stop the progression of neurovascular-related diseases and retinal degeneration by targeting ROS production and antioxidant enzyme activation.

Proteomic analysis of mouse plasma after space flight with the Bion-M2 Mission
(NASA Proposal #18-BM2-Ph2-0016)

PI Flight Investigation

PI: Kanokporn Rithidech, Ph.D., State University of New York, Stony Brook



Previous spaceflight research has shown a dysregulation in both embryonic and somatic stem cells proliferation, differentiation, and regeneration capacity in microgravity conditions, which may be the cause of the widespread tissue degeneration observed in space. However, the molecular mechanisms activated by spaceflight conditions in stem cell populations are not completely understood. This is in part because the complex and heterogeneous nature of the bone marrow compartment prevents changes in whole tissue genomic patterns from being attributed to specific sub-populations. In this investigation, we seek to understand and characterize the microgravity-induced molecular genomic changes that occur at the single cell level enabling the identification and characterization of individual cell and molecular responses to the spaceflight environment and provide insight into the molecular basis for the observed shifts in cellular phenotypes.

Objective:

- Test the hypotheses that there are specific patterns of protein-expression profiles in plasma, proteins which undergo qualitative or quantitative changes as a function of time post-landing can be identified as potential or known candidates for functional roles in response to spaceflight, patterns of global and quantitative phosphorylation status are dependent on time post-landing, and high levels of expression of proteins related to immune dysregulation and oxidative stress are associated with the severity of biological changes due to spaceflight and re-entry into the 1 g environment.
  - SPECIFIC AIM 1: Characterize the pattern of protein-expression profiles in blood plasma collected from mice flown with the Russian Bion-M2 mission at the various post-flight times: 1 hr, 12 hr, 5 d, and 15 d.
  - SPECIFIC AIM 2: Identify proteins that are candidates for functional roles in response to space flight or in re-adaptation to the 1 g environment.
  - SPECIFIC AIM 3: Gain global and quantitative phosphoproteomic perspectives of mouse plasma collected at different times post-landing (i.e.1 hr and 15 d) relative to those of vivarium and flight hardware-housed control mice.
  - SPECIFIC AIM 4: Determine a correlation between biological changes (e.g. in the cardiac/vascular system, central nervous system, muscles/bone, and immune system) and a specific pattern of proteomic status.

Experimental Approach – Following recovery from the Bion-M2 capsule, mice will be euthanized at 4 post-landing timepoints; 1 hour, 12 hour, 5 days, and 15 days. Approximately 200 µL of plasma will be collected per timepoint for proteomic profiling, phosphoproteomics, and validation of the findings (by means of western blotting or ELISA).

Relevance/Impact – The shift of global molecular signatures on *in* vivo responses in samples collected at different times after landing may provide superior biological markers for the effects of exposure and re-adaptation to gravity. Hence, it is important to conduct studies using “omic” technologies to discover the effects of space flight and the mechanisms of re-adaptation to the earth gravity (1 g environment). This study addresses Space Biology Science Plan's AH5, AH10, AH14, and CC8.

Exploration benefit – Few studies have investigated the proteomic profiling of blood plasma collected from astronauts/cosmonauts. Proteomic results from this study, in combination with the physiological/functional and clinical data for each mouse, will help address the potential biomarkers for biological/physiological dysfunction (including immune dysfunction) induced by the space environment and for readaptation to the 1 g environment.

Earth benefit – Observed proteome changes will be assessed in the context of known interactome systems to obtain a better understanding of possible alterations in broader biological and biochemical processes, which may lead to clinical relevance as a prognostic or diagnostic tool for Earth-based immune dysfunctions.

Re-adaptation after spaceflight: mechanisms and impact on long-term tissue health

(NASA Proposal #18-BM2-Ph2-0017)

PI Flight Investigation

PI: Candice Tahimic, Ph.D., KBRWyle

The gut microbiome is important in regulating human health, exerting its effects on tissues by regulation pathways involved in inflammation, aging, and immune responses. This investigation will focus on the role of gut microbiome in altering host bone function in response to spaceflight. The overarching goal is to gain mechanistic insight into how the gut microbiome can impact tissue health by modulating pathways relevant to skeletal responses to spaceflight and the ensuing recovery. Results will provide significant advances in understanding the gut microbiome in mediating adaptations to spaceflight because it will be one of the first to test the direct link between spaceflight microbiome and changes in functional outcomes in multiple tissues by microbiome transfer in mice.

Objective:

- Test the hypothesis that spaceflight leads to deficits in gut integrity and altered microbiome composition which can promote host immune and inflammatory responses and reduce osteoanabolic signaling; Recovery from spaceflight involves the restoration of normal microbiome balance, resolution of host inflammatory and immune responses and the enhancement of IGF1 signaling.
  - SPECIFIC AIM 1: Define spaceflight-mediated changes in gut integrity and microbiome composition and their impact on host inflammatory responses.
  - SPECIFIC AIM 2: Determine the contribution of the gut and its microbiome to bone homeostasis.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice (3-8 months old at launch) will be euthanized and dissected at 3 post-landing timepoints; 1-3 hours, 14 to 17 hours, and >7 days. The following tissues will be collected and frozen or fixed: gut (colon), feces, bone (femur marrow, femur, tibia, vertebrae or ischium) and serum. Changes in the integrity of the gut will be assessed on the basis of structure (histology, immunohistochemistry) including morphology of intestinal crypts and mucosal thickness. Neutrophil infiltration will be evaluated histologically, LCN2 immunostaining in intestinal epithelium and fecal LCN2 will be measured, in addition to serum analysis for host inflammatory responses and loss of gut integrity.

Relevance/Impact – This investigation will address the knowledge gap pertaining to the consequences of spaceflight-induced microbiome changes on tissue function and signaling. Microbiome studies performed to date have been limited in number and focused on defining changes in microbe populations during flight and return to Earth, but do not reveal the consequences of spaceflight-altered microbiome on host physiology. The study addresses the following Space Biology guiding questions: CMB-1, 3, 5, 7 and 8, AN-1, and MB-1, MB-5, MB-7 and the following risks and gaps identified by the HRP Roadmap: (1) Risk of Adverse Health Effects Due to Host-Microorganism Interactions: Micro-04 and Micro-05; (2) Risk of cardiovascular disease and other degenerative tissue effects from radiation exposure and secondary spaceflight stressors: Degen-2, 6, and 7 and (3) Risk Of Early Onset Osteoporosis Due To Spaceflight: Osteo-4, 6 and 7.

Exploration benefit – This novel study will significantly advance understanding of the importance of the gut microbiome in mediating adaptations to spaceflight because it will test the direct link between spaceflight microbiome and changes in functional outcomes in multiple tissues (bone, blood, gut). Results from this study may identify candidate targets (e.g. microbiome, IGF1 signaling) for maintaining skeletal health during and after extended spaceflight missions.

Earth benefit – The proposed work has translational relevance for the possible development of non-invasive and sensitive biomarkers in Earth-based diseases, such as irritable bowel syndrome (IBS) or for development of probiotic-based countermeasures.

Time course for re-adaptation of thermoregulation and bone following spaceflight

(NASA Proposal #18-BM2-Ph2-0023)

PI Flight Investigation

PI: Russell Turner, Ph.D., Oregon State University

Spaceflight results in increased fat infiltration into bone marrow and decreased hematopoiesis and bone loss. Thermo-regulation is critically important to maintain core body temperature within a narrow range. The sympathetic and sensory nervous systems regulate non-shivering thermogenesis in brown adipose tissue (BAT). Chronic increases in non-shivering thermogenesis in response to increased sympathetic outflow from the hypothalamus lead to replacement of hematopoietic tissue in bone marrow with white adipose tissue (WAT) as well as bone loss.

Objective:

- Test the hypothesis that increased non-shivering thermogenesis induced by elevated sympathetic signaling contributes to increased bone marrow adiposity, decreased hematopoiesis and bone loss in mice during spaceflight.
  - SPECIFIC AIM 1: Hypothalamus: Determine the effects of spaceflight and re-adaptation on expression of genes related to sympathetic outflow.
  - SPECIFIC AIM 2: Brown adipose tissue (BAT): Determine the effects of spaceflight and readaptation on BAT histology and expression of genes related to non-shivering thermogenesis.
  - SPECIFIC AIM 3: White adipose tissue (WAT): Determine the effects of spaceflight and readaptation on WAT histology and expression of genes related to WAT turnover.
  - SPECIFC AIM 4: Marrow adipose tissue (MAT): Determine the effects of spaceflight and readaptation on bone and MAT histology, lipid composition of bone marrow and expression of genes related to bone cell and adipocyte turnover and hematopoiesis.

Experimental Approach – Following recovery from the Bion-M2 capsule, C57Bl/6 male mice will be euthanized and dissected at 4 post-landing timepoints; 3 hours, 12 hours, 5 days, and 15 days. The following tissues will be collected and frozen or fixed: hypothalamus, interscapular BAT, intraperitoneal WAT, bone from appendicular skeleton (e.g. tibia or femur) and bone from axial skeleton (e.g. lumbar vertebra). Histology analysis, lipid composition of MAT, gene expression and bone microarchitecture will provide insight into the specific pathways by which altered thermogenesis influences bone metabolism during spaceflight and during re-adaptation.

Relevance/Impact – By altering the differentiation program of stem cells residing within bone marrow, spaceflight results in increased bone resorption and bone loss, increased bone marrow adiposity, anemia and impaired immune function. Because these disturbances may potentially compromise the success of long-duration missions, there exists an urgent need to identify the underlying mechanisms and implement effective countermeasures. This study addresses Space Biology Guiding Question QCB-2: How do changes in gravity affect the regulatory mechanisms that govern alterations in the musculoskeletal system in animals and QCB-3: Do changes in microgravity affect the basic metabolic rate and metabolism of living systems.

Exploration benefit – Studies to date address the role of increased MAT in influencing hematopoiesis and bone balance during spaceflight but have not addressed the underlying cause for fat infiltration into the marrow cavity. Results from this investigation will elucidate the relationship between bone marrow adiposity and bone formation, which is crucial for maintaining astronaut health during long-duration missions.

Earth benefit – Regulation of energy metabolism involves the integration of signals from the digestive system, pancreas, liver, adipose tissue, thyroid, bone, hypothalamus and pituitary. There is accumulating evidence that nutritional deficiencies may contribute to the detrimental effects on the bone marrow microenvironment. Results from this study may lead to nutritional, energy-intake balanced countermeasures to alleviate bone marrow and skeletal deficiencies.

PI Requests

This table outlines the PIs' requests as stated in the grant. Further discussions are required to finalize the details.

Principal Investigator	Strain	Age	Sex	Number of Mice	Timepoints / # of animals
Blaber	C57BL/6	4-5 months	male	40	1-3 hr / 10 12-14 hr / 10 5 days / 10 15 days / 10
Bouxsein	C57BL/6	Did not specify	male	24	1-3 hr / 6 15-17 hr / 6 5 days / 6 15 days / 6
Delp	C57BL/6	Did not specify	male	54	14-17 hr / 18 5 days / 18 15 days / 18
Holets	C57BL/6	45-60 days	male	6-8	15 days / 6-8
Lee	C57BL/6	5 - 6 months	male	40	1 hr / 10 12 hr / 10 5 days / 10 15 days / 10
Mao	C57BL/6	9 wks	male	18-24	< 17 hrs / 6- 8 < 72 hr / 6-8 3-15 days / 6-8
Rithidech	C57BL/6	Did not specify	male	24	1 hr / 6 12 hr / 6 5 days / 6 15 days / 6
Tahimic	C57BL/6	4-5 months	male	30	1-3 hr / 10 14-17 hr / 10 > 7 days / 10
Turner	C57BL/6	4-5 months	male	40	3 hr / 10 12 hr / 10 5 days / 10 15 days / 10

PI Tissue Requests

Tissues	Blaber	Bouxsein	Delp	Holets	Lee	Mao	Rithidech	Tahimic	Turner
Aorta			Aorta						
Basal veins			Basal veins						
Basilar artery			Basilar artery						
Blood	Blood	Blood (serum)	Blood (plasma)	Blood	Blood (serum and whole)		Blood (plasma)	Blood (serum)	
Brain			Brain			Brain			Hypothalamus
Brown Adipose Tissue (BAT)									BAT
Cerebral arteries			Cerebral arteries						
Cervical lymphatics			Cervical lymphatics						
Choroid plexus			Choroid plexus						
Colon								Colon	
Coronary arteries			Coronary arteries						
Cranium	Cranium (bone marrow)								
Epididymis				Epididymis					
Eyes						Eyes			
Feces		Feces						Feces	
Heart			Heart		Heart (left ventricular portion)				
Humerus	Humerus (bone marrow)								
Jugular veins			Jugular veins						
Liver	Liver								
Ophthalmic arteries			Ophthalmic arteries						
Pelvis	Pelvis (bone marrow)							Ischium	
Rib	Rib (bone marrow)								
Spleen	Spleen								
Soleus		Soleus							
Testis				Testis (and sperm)					
Tibia	Tibia (bone marrow)	Tibia bone (right)						Tibia	Tibia (marrow or similar)
White Adipose Tissue (WAT)									WAT
Vertebrae	Vertebrae (thoracic) (bone marrow)							Vertebrae (lumbar)	Vertebrae (lumbar)

This table outlines the PIs' tissues requests as stated in the grant. The tissues marked in green have multiple requests and further discussions are required to finalize the details.