Evidence Report

RISK TITLES:

RISK OF IMPAIRED PERFORMANCE DUE TO REDUCED MUSCLE MASS, STRENGTH &, ENDURANCE (SHORT TITLE: MUSCLE)

AND

RISK OF REDUCED PHYSICAL PERFORMANCE CAPABILITIES DUE TO REDUCED AEROBIC CAPACITY (SHORT TITLE: AEROBIC)

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Human Research Program
Human Health Countermeasures Element
Approved for Public Release: Month DD, YYYY

National Aeronautics and Space Administration
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Status:

- *Active*: Work/research is currently being done towards this risk
    - Spacephys
    - ZeroT2
    - Egress fitness
    - Fitness for duty

Executive Summary

This report reviews the scientific literature regarding the human system risks to the microgravity environment of space flight in relation to human performance. The primary human performance-related risks involve deconditioning of the cardiovascular and skeletal muscles systems due to prolonged exposure to the reduced gravitational input. The chronological history of U.S. space flight is reviewed as a starting point to inform and understand the gaps in the knowledge to these risks.

Maintenance of physical performance capabilities involves understanding the health of many organ systems (peripheral [vascular, heart, blood volume, skeletal muscle] and central [brain]) that ultimately contribute to the submaximal and maximal capacity of the aerobic (VO_2peak), skeletal muscle (strength and endurance) systems. Maintaining astronaut VO_2peak, muscle mass, strength, and endurance before, during, and after space flight is a significant priority to NASA for the current International Space Station (ISS) era, as well as for future exploration missions. A growing research database from both space flight and ground-based analog studies finds that the cardiorespiratory system is compromised and skeletal muscles (predominantly postural muscles of the lower limbs) undergo atrophy. These structural and metabolic responses to living in microgravity conditions contribute to physiological deconditioning during space flight that potentially increase the risks to astronauts returning to surface operations (i.e., Moon, Mars, or Earth). The time course changes from short to long-duration space flight and the relationships between in-flight performance deconditioning levels are not well characterized. Moreover, there are large interindividual variabilities that may be dependent on genetics, age, sex, preflight fitness levels, and individual exercise prescriptions that need further careful evaluations. Efforts should be made to understand the current status of preflight, in-flight, and postflight exercise performance capability and to define the operational goals and target areas for protection with the in-flight exercise program.

There is a bi-directional relationship between exercise prescription and hardware countermeasures that need further understanding in-flight. For example, hardware with limited capabilities/modalities may be counterbalanced by changes in exercise prescription (i.e., frequency, time, intensity, volume) for providing effective responses to maintain fitness. Importantly, the minimal requirements for exercise prescription on ISS hardware may not translate to lower capability hardware on exploration missions. Due to limited volume on exploration vehicles, future Artemis missions to the Lunar surface will not have similar exercise hardware

capabilities as ISS. This may alter the effectiveness of hardware to provide adequate physiological stress on bodily systems allowing for adaptations to maintain aerobic capacity, strength, and bone density. Thus, it will be important to understand the exercise responses of current ISS countermeasures to develop individualized exercise prescriptions that minimize aerobic and muscular risks, accounting for the large variability of responses among crewmembers. Newer exploration exercise hardware is currently being evaluated that is more compact (i.e., E4D and Orion Flywheel) and will require careful evaluation of the hardware on the stressor (i.e., metabolic rate, oxygen uptake, and heart rate work relationships, and force plate load profiles) needed the human body to protect and maintain crew health and performance. Moreover, exercise responses on the hardware need careful evaluation on the chronic adaptations. Lastly, in-flight evaluation of hardware exercise response may differ in 0-g or partial-g compared to 1-g. Therefore, it cannot be assumed that the stress on the body will be the same in each environment. Understanding this has a direct impact on exercise prescriptions.

This document provides an overview of key scientific investigations that have been conducted before, during, and after human space flight missions, as well as from human ground-based analog studies that contribute to the evidence base on changes in aerobic capacity and muscle mass, strength, and endurance. Additional data from rodent and nonhuman primate experiments of skeletal muscle unloading completed during space flight or ground-based flight-simulations provide supportive information about this risk topic. Most importantly, a recent, large dataset from long-duration ISS crew has been added to give improved insight into the variability of exercise response of crew, demonstrating that a large portion of the crew population return to Earth with greater than 10-20% loss of aerobic capacity and muscle strength and endurance. Data from human space flight and ground-based studies are narrowing in on the required exercise paradigms but thus far still provide an incomplete answer to an effective approach for maintaining skeletal muscle function and aerobic fitness of all human space travelers. Finally, the relationship of this risk topic to various space flight operational scenarios is examined and discussed.

1. EVIDENCE

a. Introduction

The change in gravity from Earth (1-g) to microgravity (0-g), prolonged exposure in 0-g, and re-entry to 1-g (or partial-g) environmental conditions over the course of a mission space flight will stress an astronaut's multi-organ systems and will result in physiological acclimations (on the order of hours to days) as well as acclimatization (days to months). Moreover, nominal or emergency egress on the Moon at 0.17-g or on Mars at 0.38-g with surface exploration are expected scenarios for future missions. The duration in microgravity or partial gravitational environments will prolong deconditioning compared to 1-g, including changes to bone and mineral metabolism [56]; skeletal muscle [57, 58]; vestibular [60], cardiovascular [61], and pulmonary systems [62]; and hematological variables [57, 58]. The changes and adaptations that occur during space flight can affect overall health and the functional exercise capacity of the astronauts [63-66]. The maximal capacity of the aerobic system and skeletal muscle strength are significantly affected by exposure to microgravity. Specifically, space flight-induced deconditioning will impact the capacity of the heart to pump and systemically deliver oxygen-rich blood, as well as the disposal and utilization of oxygen that ultimately have an impact on skeletal muscle oxidative capacity and cardiorespiratory fitness. Space flight-induced deconditioning will also result in muscle structural (i.e., atrophy) and metabolic alterations that ultimately lead to decrements in muscle strength and endurance, fatigue resistance, motor performance, and connective tissue integrity [67].

Physical movements require an integration of all organ systems; thus, maintaining overall health is needed to effectively complete long-duration and strenuous physical tasks to successfully fulfill mission objectives (i.e., extravehicular activities, EVAs) and return to Earth safely (i.e., egress). Most importantly, an individual's maximal capacity of the cardiorespiratory system and skeletal muscle, strength, and endurance determines the ability of astronauts to complete submaximal and prolonged aerobic/endurance physical work and short bursts movements. From an engineering perspective, accurate measurement of an astronaut's oxygen uptake data is also important. Knowing the energy expenditure during physical activity, and the submaximal oxygen utilization, carbon dioxide production, and the metabolic heat production and dissipation are vital for spacecraft Environmental Control and Life Support System (ECLSS) designs, including the EVA suits. This normal adaptive response to the microgravity environment is, for the most part, of little consequence within the space vehicle per se but may become a liability resulting in increased risk of an inability or decreased efficiency in crewmember performance of physically demanding tasks during EVA or upon reintroduction to transitions to partial-g and 1-g environments (such as return to Earth or landing on the surface of another planetary body).

For example, EVA and emergency egress actions upon landing on Earth or during future exploration missions will require maintenance of crew health and physical function [68, 69]. In these scenarios, the crewmembers are under high levels of stress while wearing space suits that restrict movement in any environment and are heavy when in a gravitational environment. Moreover, emergency egress tasks require normal ambulatory subjects to work at moderate exercise intensities (e.g., 85% of maximum heart rate, HR) [70] and would be a much greater challenge after long-duration ISS missions that typically last at least 6 months. During lunar EVAs conducted during the Apollo era, there are several reports of EVAs performed at 78-85% of maximum HR [71]. Although the precise plans regarding space exploration destinations are not well-defined, future exploration activities may require higher levels of aerobic fitness compared to the ISS microgravity EVA task requirements. Notably, a deconditioning in VO_2peak of 10% could severely limit the ability to perform activities requiring 85% of preflight VO_2peak. It is critical to maintain cardiorespiratory fitness and strength during long-duration space flight so that crewmembers can effectively complete long-duration and strenuous physical tasks that are required of them.

In the U.S. human space program, the only in-flight countermeasure to prevent the reduction in aerobic capacity and strength is prescribed exercise. In-flight exercise hardware and exercise prescription protocols have varied and depend on mission duration and the volume of the spacecraft available for performing countermeasures. Notably, long-duration missions and exploration missions with several transitions between gravitational environments (1-g to 0-g to 0.3-g to 0-g to 1-g) present the greatest challenges to risk mitigation. Russian scientists have utilized a variety of exercise hardware and in-flight exercise protocols during long-duration space flight (up to and beyond 1 year) aboard the Mir space station. On ISS, a combination of resistive and aerobic exercise has been used for missions of various duration, including beyond 1 year [7, 52]. Outcomes have been acceptable according to current expectations for crewmember performance on return to Earth; however, there still is large variability of fitness from pre to post space flight that can be improved upon. Moreover, for missions to the Moon, establishment of a lunar base, and interplanetary travel to Mars, the functional requirements for human performance during each specific phase of these missions have not been sufficiently characterized.

The purpose of this document is to provide a review of historical and current relevant data that relate to the risk of impaired performance due to reduced aerobic capacity and muscle mass, strength, and endurance in response to human space flight. Table 1 provides a description of the terminology used frequently through this document to address the associated risks.

Table 1. *Key terms, definitions, and associated risks*

Term(s)	Definition	Integrated Organ System Risks	Human Performance Risk
Aerobic Capacity (VO_2peak)	Maximum amount of oxygen that the body can use during maximal exercise that involves large muscle groups at a moderate to high intensity for prolonged periods	Structure and function of the circulatory, vascular, respiratory, and pulmonary systems	Physical capacity of submaximal and maximal aerobic system to complete emergency egress during landing and in partial gravity that allows for meeting the demands of performing mission-specific activities of varying intensities for extravehicular and daily activities, EVA in-suit risks for fatigue, hypercapnia, and thermal strain in planetary environments (e.g., Lunar and Martian surfaces), psychological readiness and performance
Muscle Mass, Strength, and Endurance	Size and strength of skeletal muscle group associated with the ability of skeletal muscles to generate force necessary for all types of movement	Structure and function of the skeletal muscle contractions, neurovestibular and motor performance (e.g., balance, coordination, spatial orientation), and skeletal muscle and tendon injury	
Exercise Countermeasures	Hardware and prescription of exercise required to provide aerobic and resistive training stimuli needed to maintain health and performance that enables successful mission task performance.	Effective hardware is needed to maintain aerobic capacity and muscle strength and endurance. Aerobic and resistance exercise are the only known and used countermeasures to reduce organ system and human performance risks.	

This comprehensive assessment aims to provide a thorough understanding of the changes that occur from reduced gravity-induced deconditioning and to guide mission-specific functional requirements. It is also important to understand the countermeasures that provide a functional maintenance level of aerobic capacity, strength, and endurance performance sufficient to maintain crew health, safety, and performance of operational tasks. In addition, crew exercise prescriptions will impact environmental control systems (in both vehicles and space suits) due to carbon dioxide (CO_2) and metabolic heat production. Conversely, vehicle design and volume will affect inclusion of exercise countermeasure capabilities that should be carefully considered for future prolonged missions. Exploration vehicles will likely be limited in volume; thus, available exercise hardware countermeasures capabilities may not provide similar robust countermeasures protection as on ISS. The planned mission objectives and contingency scenarios help define the risks that must be mitigated. Evidence from over six decades of human space flight experience indicates that there are still gaps in our knowledge and in our current approach to mitigate all

human system risks. Although improvements have been made in the ability to maintain crewmember aerobic capacity, strength, and endurance, eliminating variability between crewmember changes in fitness and preservation of an appropriate level in every crewmember has not yet been achieved.

b. Human space flight

Most of the observations and research studies on aerobic capacity, muscle mass, strength, and endurance during and after space flight reviewed in this section are derived from experiences in the U.S. space program.

i) Program Era

Prior to launch of the first American astronaut, suborbital flights of non-human primates (chimpanzees) demonstrated that launch, entry, and short-duration microgravity exposure were all survivable events [45] . The initial biomedical selection criteria for the first group of astronauts were established in Project Mercury (1959–1963). Medical requirements for the Mercury astronauts were formulated by the NASA Life Sciences Committee, an advisory group of distinguished physicians and life scientists. Final selection criteria included results of medical testing as well as the candidates' technical expertise and experience. Aeromedical personnel and facilities of the Department of Defense (DoD) were summoned to provide psychological and stress testing of astronaut candidates. The screening and testing procedures defined for the selection of Mercury astronauts served as the basis for subsequent selection of Gemini and Apollo astronauts. Notably, only male candidates were selected. While a group of female pilots underwent the same screening and testing procedures, but the program ultimately lost funding. Screening health and performance evaluations included exercise test of aerobic fitness and strength have been performed since the beginning of manned space flight. Early tests consisted of rudimentary evaluations of aerobic exercise tolerance and strength. Over time, testing techniques and protocols were improved with standard measures of VO_2peak and muscle strength during the ISS era.

c. Project Mercury

The success of the suborbital flight of Alan B. Shepard in the Mercury space capsule "Freedom 7" on May 5, 1961, marked the beginning of manned exploration of space by the U.S. Project Mercury was conducted using small vehicles capable of holding only one occupant. Early astronaut selections (Mercury–Apollo) were exclusively male. These early flights were conducted with the objectives of 1) orbiting a manned spacecraft around the Earth, 2) investigating man's ability to function in space, and 3) demonstrating the successful recovery of both man and spacecraft safely [45]. While the Mercury flights were largely demonstration flights, the longest Mercury mission being only about 34 hr, Project Mercury clearly demonstrated that humans could tolerate the space flight environment without major acute physiological effects and some useful biomedical information was obtained, including the following [45]:

- Pilot performance capability was unaltered by space flight. All measured physiological functions remained within acceptable normal limits.
- No signs of abnormal sensory or psychological responses were observed.
- The radiation dose received was considered insignificant from a medical perspective.
- Immediately after landing, an orthostatic rise in heart rate and drop in systemic blood pressure were noted, which persisted for 7 to 19 hr post landing.

i) Aerobic Capacity, and Muscle Mass, Strength, & Endurance

Table 2. *Physical characteristics from candidates and selected Mercury Project Crew*

Physiologic Data				
	Astronaut candidates (31)		Astronauts selected (7)	
Test	Mean	Range	Mean	Range
Height (cm)	176.0	167–180	177.0	177–180
Weight (kg)	73.4	61.87	75.3	70–87
Body surface area (m2)	1.9	1.7–2.1	1.9	1.8–2.1
Lean body mass (kg)	63.9	55–71	66.8	59–71
Total body potassium (gm)	168.6	142–204	175.4	167–199
Total body water (L)	41.3	36–47	41.5	37–45
Blood volume (L)	4.9	3.33–6.91	5.4	4.35–6.91
Total circ. Hemoglobin (gm)	756.5	565–1,127	857.2	674–1,120
Total lung capacity (L)	6.8	5.36–8.19	7.0	6.34–8.02
Functional residual capacity (L)	3.2	2.25–4.23	3.4	2.96–4.23
Vital capacity (L)	5.5	4.35–6.91	5.5	5.11–6.02
Residual volume (L)	1.3	0.83–2.00	1.5	1.13–2.00
Maximum breathing capacity (L)	180.0	149–247	191.0	156–247
Nitrogen clearance equivalent	11.1	9.3–13	10.9	9.2–12.0
Final O2 uptake during exercise (l/min)	2.4	1.90–2.84	2.6	2.07–2.84

From reference [45]

Because of the short mission durations of Project Mercury, there was little concern about loss of cardiovascular and musculoskeletal function. However, the selection criteria did ensure that astronauts were in excellent physical condition before flight. During the six flights of the Mercury program, two suborbital and four orbitals, no studies of pre- to postflight aerobic capacity and strength were conducted. However, evaluations of aerobic capacity have been essential to quantifying crew physical capacity since the beginning of the U.S Space Program (Table 2). During the selection phase for Project Mercury astronauts, potential crew underwent “physical conditioning and cardiopulmonary competence testing” [45]. Aerobic work capacity, along with other metrics of physical capacity, was measured during the final work stage of the cycle test. The exercise protocol was a graded exercise test conducted on Dobeln's bicycle ergometer that increased the load from 300 mkg/min to about 1,200 mkg/min under electrocardiographic monitoring for possible abnormalities at maximum effort. The test proceeded until the heart rate reached 180 beats/min or until signs of approaching overload were evident. The heart rate, blood pressure, respiratory volume, and respiratory gas exchange were measured each minute. Oxygen consumption attained during the highest workload was the criterion of aerobic work capacity; however, this is not considered maximal exercise.

ii) Exercise Countermeasures

During the orbital flights, the first exercise test conducted in the spacecraft was a 30-s exercise session using a bungee cord with a 16-lb pull through a distance of 6 inches [72]. The

crewmembers' HRs were increased during exercise and rapidly recovered afterwards. These were the first demonstrations that the cardiovascular system is reactive to exercise during space flight. Although exercise intolerance was not observed, in-flight exercise training was recommended for crewmember protection during future space flight missions. The following statement was included in the post-mission report of the third U.S. manned orbital flight, indicating that exercise was being considered by NASA as a possible countermeasure for space flight exposure as early as 1962 [73]:

> *An orthostatic rise in heart rate, fall in systolic blood pressure, and maintenance of the diastolic pressure was noted during the 24 hours immediately after landing. Such a hemodynamic phenomenon may have more serious implications for a longer mission. A prescribed in-flight exercise program may be necessary to preclude symptoms in case of the need for an emergency egress soon after landing.*

d. Project Gemini

Biomedical information acquired during the Mercury flights provided a positive basis to proceed with the Gemini Program, which took place during the 20 months from March of 1965 to November of 1966. The Gemini project was conducted using two-man space capsules launched to orbit atop modified U.S. Air Force Titan-II intercontinental ballistic missiles. The primary purpose of the Gemini Program was to achieve a high level of operational confidence with human space flight. These flights were conducted to gain experience necessary to conduct future missions to the moon. Therefore, the objectives of these flights were to 1) develop a better understanding of how humans tolerate extended periods of weightlessness flight exposure, 2) subject man to EVAs, 3) validate human life support systems and astronaut performance capabilities after living in space flight environment for up to 2 weeks, and 4) practice docking operations, refining the landing methodology with orbiting target vehicles [13].

In total, there were 12 Gemini flights, including two unmanned flights and 10 flights with 2-person crews. The Gemini Program resulted in about 2000 man-hours of weightless (0-g) exposure of U.S. astronauts. In the 14-day Gemini VII flight, salient observations were undertaken to further examine the physiological and psychological responses of astronauts as a result of exposure to space flight and the associated microgravity environment.

i) Aerobic Capacity

The first pre- and postflight assessment of aerobic capacity occurred in the Gemini program and used graded exercise tests on six crewmembers to determine the effects of microgravity on 14-day flight performance [13, 15]. The test protocol was conducted on an electronic cycle ergometer on which the crewmember pedaled at 60-70 revolutions per min. The work rate was set initially at 50 W for 3 min and increased by 15 W each minute until the crewmember's HR reached 180 beats·min^{-1}. The results from these tests were presented in the NASA Gemini Summary Conference Report [13]. It was reported that all but one of the crewmembers had a decrease in exercise tolerance. The decline in exercise tolerance was described as an increase in the HR response to exercise and a reduction in VO_2 at exercise termination. For example, the VO_2 at test termination was 19% and 26% lower after flight in the two crewmembers of Gemini VII [15]. Figure 1 is an illustration of the graded exercise test results of a Gemini IX crewmember [13]. It is important to note that these were not valid measurements of VO_2peak and not a true VO_2max since final measures during the test were not obtained at maximal exercise. The investigators suggested that these data provide strong evidence that aerobic capacity was compromised following the Gemini flights. The pre- to postflight decline in VO_2 at test termination

was suggested to have been related to decreased total blood volume (reduced in five of six crewmembers examined), plasma volume (decreased in four), and red cell mass (decreased in all six crewmembers). The factors that were speculated to cause these hematological changes were hyperoxia (the Gemini space craft environment was 100% oxygen at 5 psia, or 259 mmHg), physical confinement of the crew, dietary factors, and weightlessness. A variation of the Harvard Step Test (an index of aerobic capacity) was used and showed that there was no indication of decrement in the physical condition. However, the workloads imposed by this test were relatively mild and the testing time duration was brief. Therefore, it is likely this test was not a specific or sensitive measure of aerobic capacity.

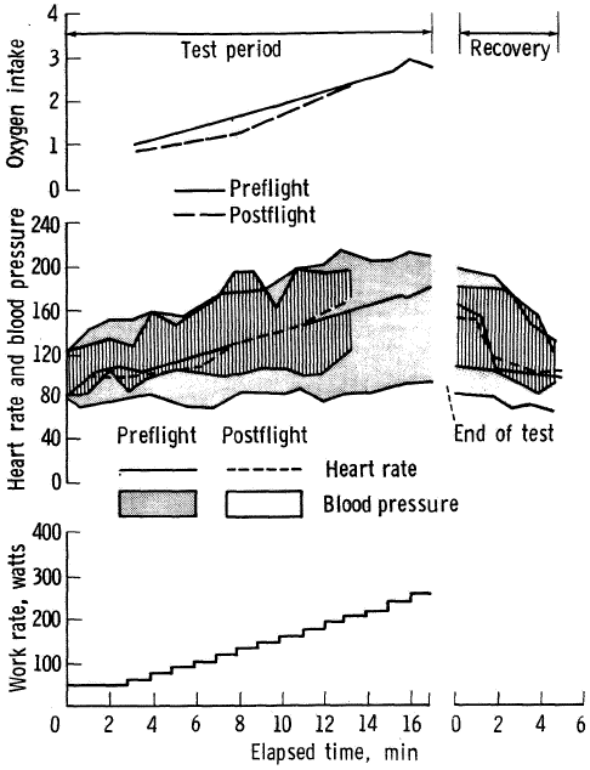

***Figure 1**. Gemini IX pre- and post-flight exercise test results. From reference [13].*

ii) Muscle Mass, Strength and Endurance

No significant decrements in performance of mission objectives were noted, and no specific measurements of muscle strength or endurance were obtained that compared preflight, in-flight, and postflight levels.

iii) Exercise Countermeasures

During three of the manned flights (Gemini IV-4 days, Gemini V-7 days, and Gemini VII-14 days), exercise testing was conducted as part of an experiment designated as M003 – In-flight Exercise and Work Tolerance [13]. These tests consisted of crewmembers performing 30-s exercise sessions with a bungee pull device (Figure 2). The target activity rate was one pull per second, and the device delivered a force of 70 lbs. (31.8 kg) at full extension. Heart rate and blood pressure were measured during these tests, which were conducted several times during each mission. Additional observations included the presence of postflight orthostatic intolerance that was still present for up to 50 hrs after landing in some crewmembers, a decrease in red cell mass of 5–20% from preflight levels, and radiographic indications of bone demineralization in the

calcaneus. The HR response of the crewmembers to the brief exercise session remained relatively constant within an individual throughout the flights (Figure 3 [15]).

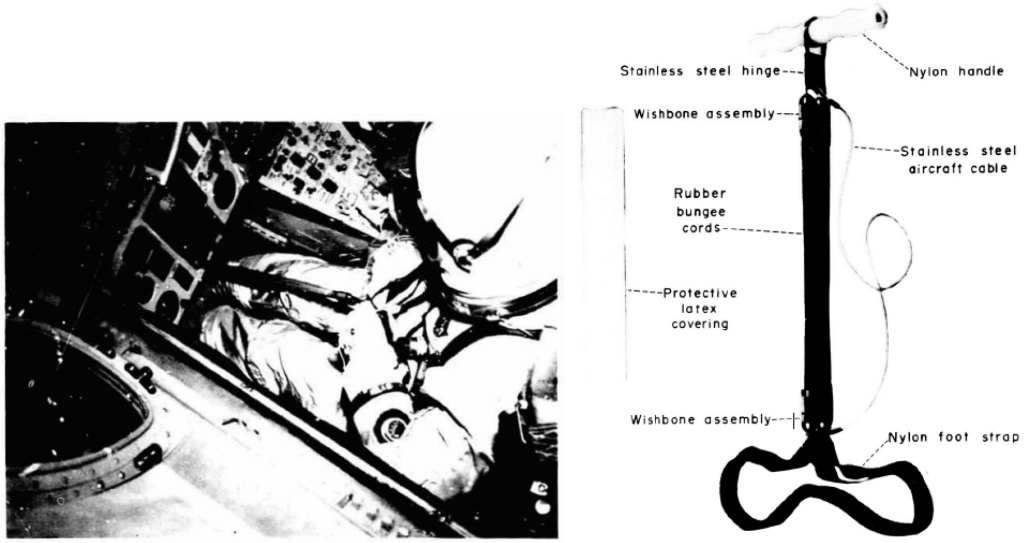

***Figure 2**. Gemini in-flight exerciser major components. Reference from [15].*

No exercise prescription countermeasures were established during these short-duration flights. Conclusions included the following:

- The response of the cardiovascular system to a calibrated workload is relatively constant for a given individual during space flights lasting up to 14 days.
- The crewmembers are able to perform mild-to-moderate amounts of work under the conditions of space flight and within the confines of the Gemini spacecraft. This ability continues essentially unchanged for missions up to 14 days.
- Using a variant of the Harvard Step Test as an index, no decrement in the physical condition of the crew was apparent during the 14-day missions, at least under the stress of the relatively mild workloads imposed in this experiment.

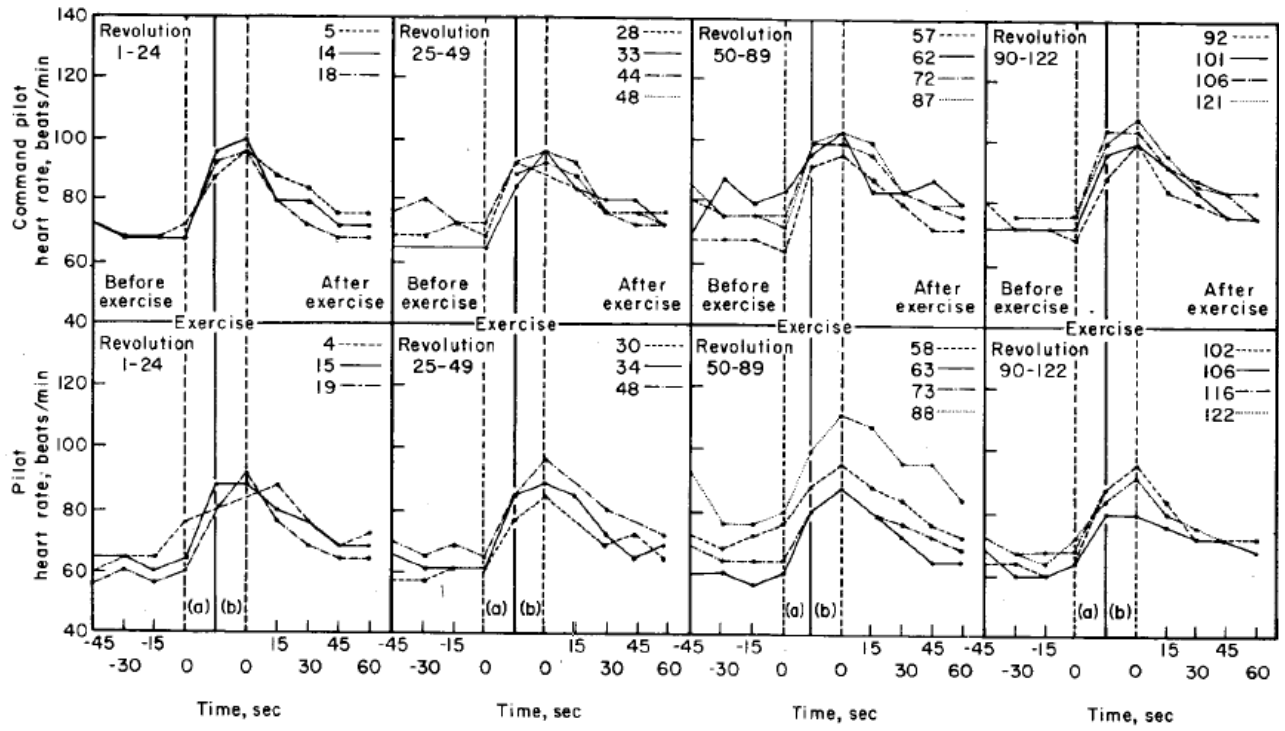

Figure 3. *Example of Gemini in-flight exercise data from two crewmembers [13].*

e. Apollo Program

The major objective of the Apollo Program was the landing of astronauts on the lunar surface and their subsequent safe return to Earth. The Apollo Program is best remembered for the flight of Apollo 11, the first manned exploration of the Moon. The Apollo Program consisted of 17 flights conducted between 1968 and 1972 [74]. Of these flights, six delivered astronauts to the Moon's surface. The Apollo crews consisted of three men per flight, and the flight durations ranged from 5.9 to 12.7 days. Biomedical results were collected from 11 crewed missions that were completed within the five-year span of the Apollo Program (pre-lunar flight missions 7 through 10), the first lunar landing (mission 11), and five subsequent lunar exploratory flights (missions 12 through 17). Apollo 13 did not complete its intended lunar landing mission because due to a pressure vessel explosion in the Service Module. Instead, it returned safely to Earth after attaining a partial lunar orbit.

Essential to the successful completion of the Apollo Program was the requirement for some crewmembers to undertake long and strenuous EVAs on the lunar surface. Concerns were identified about the capability of crewmembers to complete the lunar surface excursions planned for some of the Apollo missions. The reduced lunar gravity (1/6-g) was expected to make some tasks less strenuous; reduced suit mobility coupled with a complex and ambitious timeline led to the prediction that metabolic activity would exceed resting levels for extended periods. Since the nature and magnitude of physiological dysfunction resulting from microgravity exposure had not yet been established (and is still not concisely defined), suitable testing was completed within the

constraints of the Apollo Program to determine if crewmember physiological responses to exercise were altered as a consequence of space flight.

Initial planning for the Apollo Program included provisions for in-flight measurements of salient parameters of concern including physiological responses to exercise. However, the fire in the Apollo 204 spacecraft (also known as Apollo 1), fatal to astronauts Gus Grissom, Ed White, and Roger Chaffee, resulted in NASA management initiating changes in the program that eliminated such prospects. Thus, investigators were left with only the possibility to conduct preflight and postflight exercise response studies and to assume that these findings reflected alterations of cardiopulmonary and skeletal muscle function secondary to microgravity exposure. The following observations were reported:

- Re-adaptation to Earth gravity begins immediately upon re-entry into the Earth's gravitational field, which likely changes key physiologic responses from their measurements during space flight.
- Crew recovery procedures introduced additional challenges to a well-controlled experiment design since Apollo crewmembers spent various amounts of time in an uncomfortably warm spacecraft bobbing in the ocean. Additionally, orbital mechanics constraints on re-entry times prevented the possibility of conducting pre- and postflight testing within a similar circadian schedule.
- The impact of these uncontrollable conditions and that of other physical and psychological stresses could not be separated from responses attributable to microgravity exposure alone. Thus, data relating to the physiological responses to exercise stress in Apollo astronauts must be interpreted within this overall context.

i) Aerobic Capacity

The crews of Apollo 7-11 and 14-17 (n=27) participated in submaximal exercise testing to quantify pre- to postflight changes in the physiological response to exercise [75-78]. An electronically-braked cycle ergometer was used for exercise testing with which work rate was controlled using a HR feed-back loop. The pre- and postflight testing consisted of graded exercise stress tests [77]. Heart rate (HR) was used for determining stress levels [79], and the same heart rate levels were used for pre- and postflight testing. VO_2 was also measured during an exercise test protocol consisting of three exercise work rates which produced HRs of 120, 140, and 160 beats·min^{-1}. The Apollo 9 and 10 crews also performed an additional stage which elicited HRs of 180 beats·min^{-1}. The entire test protocol was conducted three times within a 30-day period before lift-off. Postflight tests were conducted on recovery (landing) day and once more at 24 to 36 hrs after recovery. During each test, workload, heart rate, blood pressure, and respiratory gas exchange (O_2 consumption, CO_2 production, and minute volume) measurements were made. For the Apollo 15 to 17 missions, cardiac output measurements were obtained by the single-breath technique [80, 81]. Arteriovenous oxygen differences were calculated from the measured oxygen consumption and cardiac output data.

The aerobic capacity data collected were voluminous and are summarized in tabular form by Rummel et al. [77]. In brief, reduced work capacity and oxygen consumption of significant degree was noted in 67% (18 of 27) of the Apollo crewmembers tested at recovery. This decrement was transient, and 85% of those tested (23 of 27) returned to preflight baseline levels within 24-36 hrs. The VO_2 at all exercise stages was significantly less on landing day (R+0) but was near preflight levels 24-26 hrs following landing (R+1; Figure 4). Exercise cardiac output (Q_c) measurements also were obtained from the crews of Apollo 15-17. Q_c was 37% lower on R+0 than it was before flight, and stroke volume (SV) was reduced from 145 ± 34 ml·beat^{-1} to 92 ± 34 ml·beat^{-1}. On R+1, SV was only 7% lower (not statistically different) than preflight [77]. The mean pre- to post-flight change in plasma volume of the Apollo astronauts was -4.4 ± 1.7% on R+0 and +4.8 ± 2.2% on R+1 [82]. The rapid normalization of the crewmembers' responses to exercise suggests that changes in plasma volume played a role in the post-flight decline in VO_2 on R+0 at the terminating workload. A significant decrement in cardiac SV was associated with diminished exercise tolerance. It was not clear whether the exercise decrement had its onset during flight. If it did, the Apollo data did not reveal the precise in-flight time course because of lack of in-flight measurement capabilities. The astronauts' performance on the lunar surface provided no reason to believe that any serious exercise tolerance decrement occurred during flight, except that related to lack of regular exercise and muscle disuse atrophy [83].

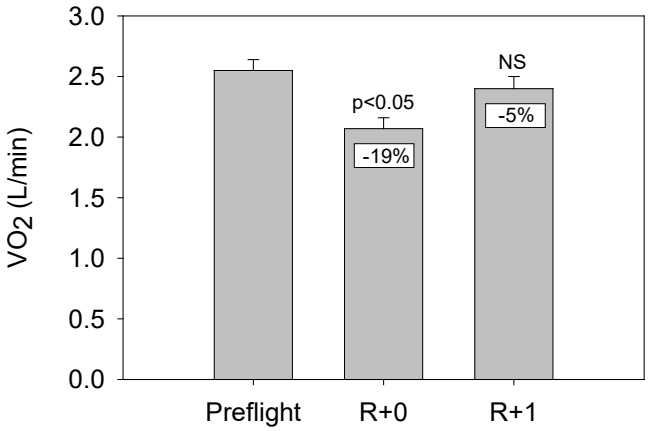

Figure 4. *VO2 changes at exercise stage eliciting a HR of 160 beats/min (Apollo crews n=27).*

ii) Muscle Mass, Strength, & Endurance

No specific measurements of muscle strength or endurance were obtained that compared preflight, in-flight, and postflight levels. Skeletal muscle atrophy is mentioned [83] with respect to its possible contribution to exercise intolerance, and in some of the later Apollo flights lower limb girth measurements were completed (data not published) that provided the first evidence for loss of muscle mass in the legs.

iii) Exercise Countermeasures

No standardized in-flight exercise program was planned for any of the Apollo flights; however, an exercise device (Figure 5) was provided on some missions. Crewmembers, when situated in the Command Module (CM), typically used the exerciser several times per day for periods of 15-30 min. The studies completed during Apollo, although less than optimal, left no doubt that a decrement in exercise tolerance occurred in the period immediately after landing, although it is believed that such decrements were not present during surface EVA. It seems likely that multiple factors are responsible for the observed decrements, with lack of sufficient exercise and development of muscle disuse atrophy as potential contributors. Catabolic tissue processes may have been accentuated by increased cortisol secretion consequential of mission stress and individual crewmember reaction to such stress. Additional factors associated with the return to Earth's gravity may also be implicated. For instance, the observed diminished stroke volume

(cardiac output) is certainly contributory and, in turn, is a reflection of diminished venous return and contracted effective circulating blood volume induced by space flight factors [83].

Figure 5. *The exercise device used on some Apollo missions was based on the Exer-Genie developed by Exer-Genie, Inc., Fullerton, CA. Within the cylinder, the nylon cords rotate around a shaft, developing controlled resistance. The cords are attached to loop handles. When not in use, the flight device was stored in a cloth bag (inset).*

f. Skylab Program

The Skylab Program (June 1972–April 1974) was the first U.S. space station and the first experience with "longer" duration space flight [74]. From the onset, Skylab was intended to provide a life sciences laboratory in space. A significant number of experiments were conducted to provide physiologic data from humans exposed to long-duration (28–84 days) stays in a microgravity environment. A 56-day ground-based simulation of many Skylab experiments, conducted in an environmentally controlled, enclosed chamber, was termed the Skylab Medical Experiments Altitude Test (SMEAT) and represented the first mission. The station was launched in May 1973 atop a Saturn V vehicle, the last launch of the rocket that first took man to the moon. Three crews traveled to Skylab using Apollo-era command modules launched on Saturn 1B vehicles. The program included one unmanned mission (Skylab 1) and three manned missions with durations of 28 days (Skylab 2), 59 days (Skylab 3), and 84 days (Skylab 4). Medical activities accounted for approximately 7% of the mission time during flight.

i) Aerobic Capacity

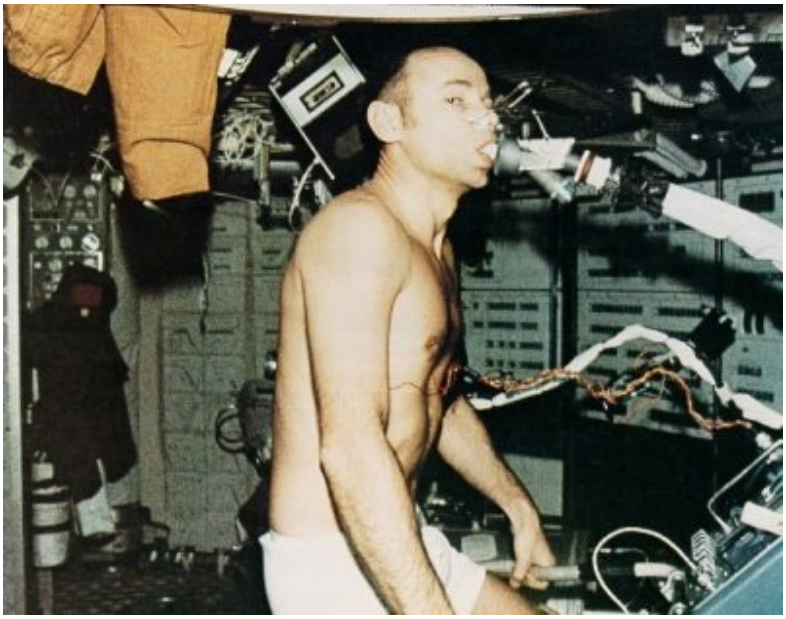

Figure 6. *Skylab crewmember performing cycle exercise test with VO_2 measurements*

The Skylab missions marked the first time that metabolic gas analysis hardware was available for use during flight. The Skylab metabolic gas analyzer is described in detail by Michel et al. [84]. During the Skylab missions, routine submaximal graded exercise testing was performed on a cycle ergometer, and expired metabolic gasses were analyzed to determine VO_2 [84, 85] (Figure 6). The submaximal exercise consisted of 50-min stages of rest followed by exercise at work rates eliciting 25%, 50%, and 75% of preflight VO_2peak. Preflight VO_2peak was established during previous graded exercise tests to volitional fatigue conducted one year (L-360) and 6 months (L-180) prior to launch. The submaximal exercise test was repeated approximately every 6 days during each flight, starting with flight day 6. The in-flight HR response to submaximal exercise did not change during the mission, which was taken as an indicator of no change in the aerobic fitness of the crews. Cardiac output (Q_c) was not measured during flight, but it was measured during the exercise tests performed before and several times following flight [86]. The mean Q_c of all crewmembers at the 75% work stage was decreased by approximately 30%, and SV was decreased by 50% on R+0. Within 10 days after landing, Q_c and SV were within 10% of preflight values, but complete recovery was not noted until 31 days following flight. The HR response to exercise was markedly elevated immediately following flight and gradually returned to preflight levels by R+24 days. Plasma volume declined by 12.5% on R+0 and returned to preflight values by R+14 days [87]. These changes did not appear to be related to mission duration. Although VO_2peak was not measured in these subjects, the post-flight exercise responses were assumed to be consistent with a decrease in aerobic capacity during the early recovery period and a gradual return to preflight levels over the month following flight.

An attempt was made to collect VO_2peak data during instrumented personal exercise sessions to near-maximum exercise levels on four crewmembers of the Skylab 3 and 4 missions [88]. However, several problems prevented accurate measurement of VO_2peak. The Skylab cycle ergometer was limited to a work rate of 286 W, and three of the four crewmembers were able to exceed this work rate during preflight testing. Therefore, these three crewmembers performed prolonged work at 286 W during flight to elicit a “maximum effort.” The limiting factor for these sessions was leg fatigue rather than a true cardiovascular maximum effort. The device that measured expired ventilation (a component of the measurement of VO_2) could only accurately measure values up to 150 $L \cdot min^{-1}$, and this level was exceeded in several tests, possibly because of the low cabin pressure of Skylab (259 mmHg). The investigators concluded that the VO_2peak

of the crewmembers was likely maintained and perhaps even increased during flight, although the measurement hardware limitations greatly cloud the interpretation of the data.

ii) Muscle Mass, Strength, & Endurance

With respect to the issue of muscle mass and function loss, two key studies were performed during the course of the three Skylab orbital missions. First, leg and arm volumes were calculated by measuring the girth (circumference) of contiguous 3-centimeter arm and leg segments, treating all the segments as a short, tapered cylinder, and then summing the segment volumes to obtain the volume of each extremity [33]. The second study included the first muscle strength measurements by means of a dynamometer [33, 34]. In addition to measurements relating directly to skeletal muscle strength and mass, indirect measurements were made that demonstrated that all Skylab crewmembers had a negative nitrogen balance [89] indicative of skeletal muscle attrition. This was also observed 10 years later in short-duration Space Shuttle crewmembers [90].

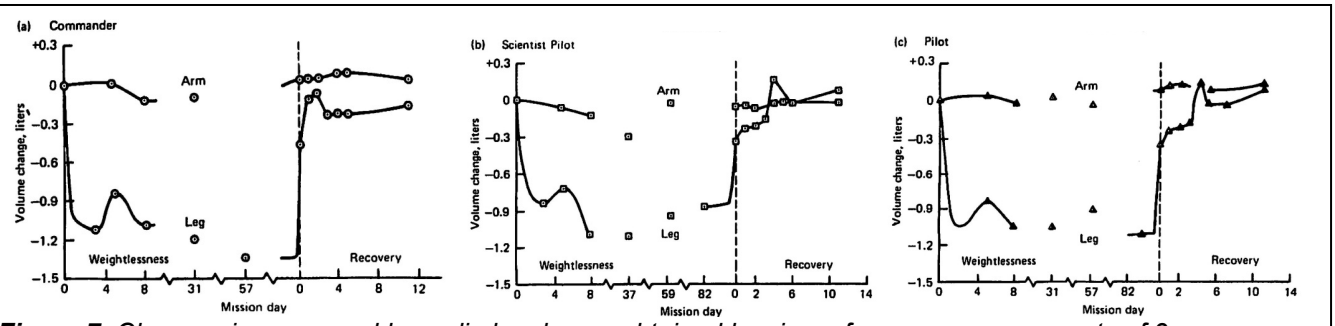

Figure 7. *Changes in upper and lower limb volumes obtained by circumference measurements of 3-cm segments in the three crewmembers from Skylab 4. It should be noted that, because of a much higher exercise volume in the Skylab 4 crewmembers, their loss of muscle volume was much less than what was observed in crewmembers from Skylab 2 and 3. From reference [33].*

Upper and lower limb volumes obtained on the three crewmembers of Skylab 4 are shown in Figure 7. Fluid shifts contributed the largest changes to lower limb volumes, but loss of leg tissue mass is evident, particularly in the Commander. As shown in the graphs, significant loss of leg volume occurs within the first few days of microgravity exposure while changes in the upper limbs are less remarkable. Upon return to Earth, much of the loss of leg volume is corrected and there is often a short overcorrection, or overshoot. Once this fluid shift resolves and slowly returns to baseline levels, the true loss of muscle mass remaining in the legs is revealed (see Figure 7, leg during recovery on right side of graph for all three crewmembers). In the Skylab 4 Commander, the loss in leg volume appears to be nearly 300 cc.

Preflight and postflight evaluation of muscle strength was performed on the right arm and leg of each crewmember for all three Skylab orbital missions by means of a Cybex isokinetic dynamometer [34]. The protocol completed by each crewmember included a thorough warm-up, and 10 maximum-effort full flexions and extensions of the arm at the elbow and of the hip and knee at an angular rate of 45°/second. The isokinetic leg strength results from all three missions, as well as body weights and leg volumes, are presented in Figures 7 and 8.

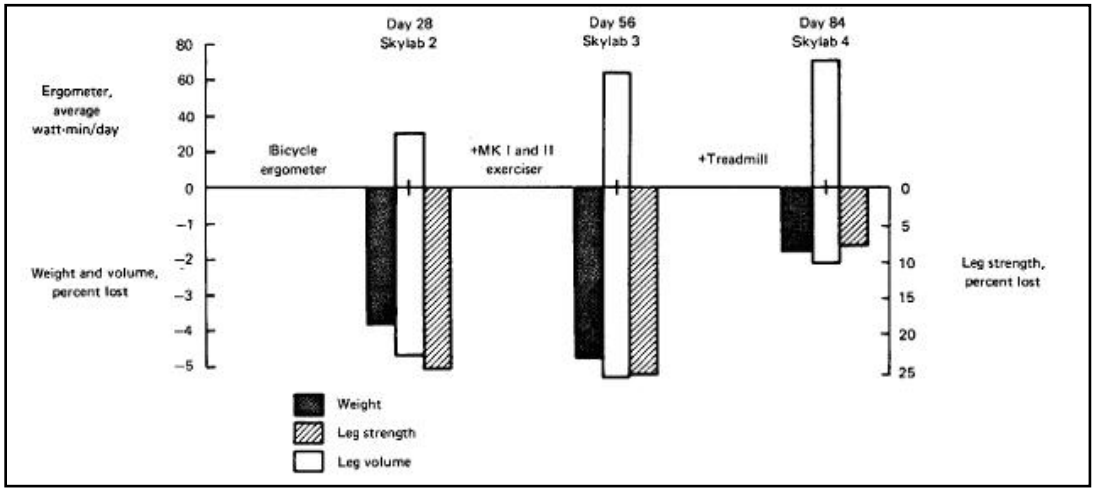

Figure 8. *Average changes in body weight, isokinetic leg strength, and leg volume of crewmembers on the three Skylab missions. Only the bicycle ergometer was used on Skylab 2, the MK I and MK II "Mini Gym" exercisers were added for Skylab 3, and a passive "treadmill" was flown on Skylab 4. The average workload per day on the cycle ergometer is also provided by mission for comparison. From reference [34].*

iii) Exercise Countermeasures

During the Skylab Program, exercises and exercise devices were added incrementally, and the testing expanded with each mission. This produced a different exercise environment for each flight so that, in reality, there were three separate but related orbital experiments, each with N = 3. The results from each mission had a significant impact on the next [34]. For instance, the complement of exercise equipment for the fourth Skylab mission was larger than the previous missions and consisted of a cycle ergometer, passive treadmill, and modified commercial devices (i.e., the "Mini gym" and MK-II) that provided the capability for low-load resistive exercises. Hence, losses in muscle mass and strength were less than in the previous two missions of shorter duration.

On Skylab 2, only the bicycle ergometer was available for in-flight exercise, with testing performed 18 days before launch and 5 days after landing. While it was realized that these times were temporally too remote from the flight, this was the best that could be achieved due to schedule constraints. By the time day 5 muscle testing was completed, some recovery in function had likely occurred; however, a marked decrement remained. The decrement in leg extensor strength was nearly 25%; the arms suffered less but also exhibited marked losses (data not shown). The Commander's arm extensors showed no loss, since he used these muscles in hand-pedaling the bicycle, being the only Skylab crewmember to adopt this mode of arm exercise. This illustrates a fundamental point in muscle conditioning: to maintain the strength of a muscle, it must be stressed to or near the level at which it will have to function. Leg extensor muscles important in standing and providing propulsive forces during walking are capable of generating forces of hundreds of pounds, while the arm extensor forces are measured in tens of pounds. Forces developed in pedaling a bicycle ergometer are typically tens of pounds and are thus incapable of maintaining leg strength. The bicycle ergometer proved to be an excellent machine for aerobic exercise and cardiovascular conditioning, but it was not capable of developing either the type or level of forces needed to maintain strength for walking under 1-g [34].

Immediately after Skylab 2, work was started on devices to provide adequate exercise to arms, trunk, and legs. A commercial device, termed "Mini Gym," (Figure 9) was modified extensively and designated "MK-I." Only exercises that primarily benefited arms and trunk were achievable with this device. While forces transmitted to the legs were greater than those from the cycle ergometer, they were still limited to an inadequate level, since this level could not exceed the maximum strength of the arms, which only represents a fraction of leg strength [34].

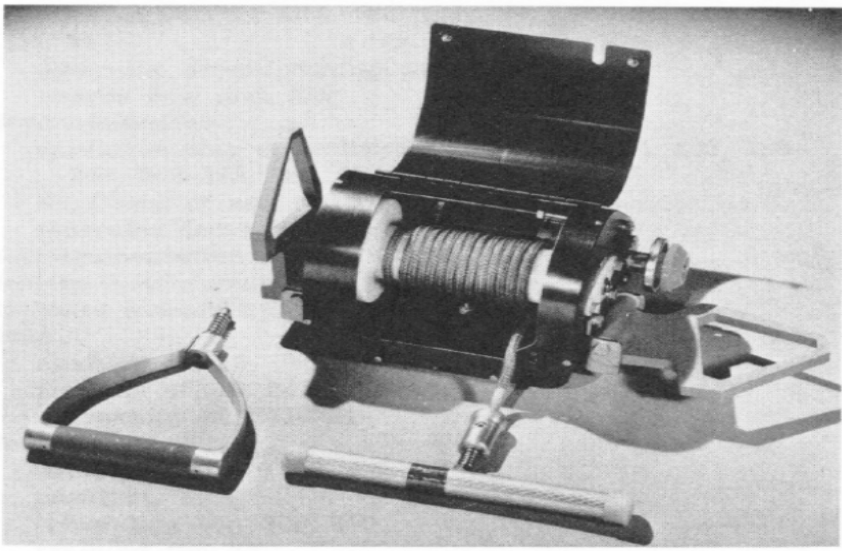

Figure 9. *The Mark I exerciser, added for the third and fourth Skylab missions, was used for a number of arm and leg exercises. This unit is a modified version of a commercial device, the Mini-Gym Model 180, marketed by Mini-Gym, Inc., Independence, Missouri. This is an isokinetic, or constant velocity, exerciser which retards the speed at which the user is allowed to move. The user applies maximum effort, and the device automatically varies the opposing resistance to maintain speed of translation at a constant preselected value.*

A second device, designated "MK-II," consisted of a pair of handles between which up to five extension springs could be attached, allowing development of maximum forces of 25 lb per foot. These two devices were flown on Skylab 3, and in-flight nutrition support and exercise time and food were increased. The crew performed many repetitions per day of their favorite maneuvers on the MK-I and, to a lesser extent, on the MK-II. Additionally, the average amount of work done on the bicycle ergometer was more than doubled on Skylab 3, with all crewmembers actively participating.

It was perceived by Skylab life scientists that a device allowing one to walk and run under forces equivalent to Earth gravity would provide more strenuous exercise [34]. Immediately after completion of Skylab 2, work began on a treadmill for Skylab 4. As mission preparation progressed, the launch weight of Skylab 4 escalated so much that the final design of the treadmill was constrained by weight limitations, the final weight being a mere 3.5 lb. This passive device (Figure 10) consisted of a Teflon-coated aluminum walking surface attached to the Skylab iso-grid floor. Four rubber bungee cords provided a weight of about 80 kilograms (175 lbs) and were attached to a shoulder and waist harness worn by crewmembers during use. By angling the bungee cords so that the user was pulled slightly forward, an equivalent to a slippery hill was created (making it necessary to wear socks with no shoes to provide a low-friction interface). High loads were placed on some leg muscles, particularly on the calf. Fatigue was so rapid the device could not be used for significant aerobic work because of the bungee/harness design.

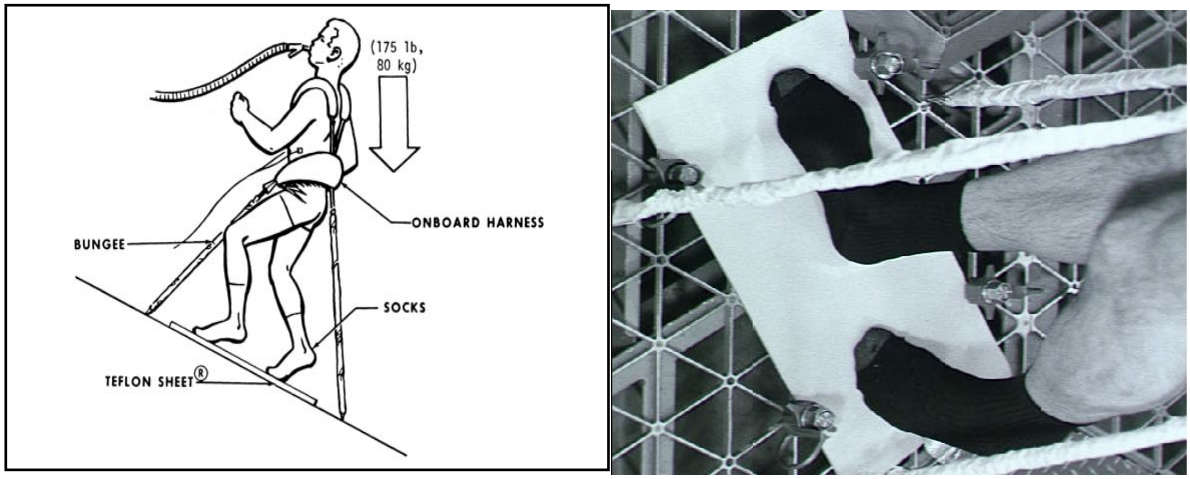

Figure 10. *The first U.S. "treadmill" utilized during spaceflight was a passive device used only on the Skylab 4 mission of 84 d duration. The high loading (175 lbs.) via bungee cords provided more of a resistive rather than an aerobic modality. It consisted of a Teflon-coated aluminum plate attached to the Skylab iso-grid floor. The exercising crewmember wore a waist and shoulder harness that attached to the iso-grid floor surrounding the treadmill plate by means of four bungee cords. Socks had to be worn to provide a low-friction interface between the plantar surface of the feet and the Teflon-coated treadmill plate. From reference [34].*

On Skylab 4, the crew used the bicycle ergometer at essentially the same rate as on Skylab 3, as well as the MK-I and MK-II Mini Gym exercisers. In addition, they typically performed 10 min per day of walking, jumping, and jogging on the treadmill. Food intake had again been increased. Upon their return to Earth and even before muscle testing, it was apparent that the Skylab 4 crewmembers were in very good physical condition. In contrast to the crewmembers from the earlier two missions, they were able to stand and walk for long periods without apparent difficulty on the day after landing (R+1). Results of strength testing confirmed a surprisingly small loss in leg strength even after nearly 3 months of microgravity exposure. In fact, knee extensor strength increased over the preflight level.

Throughout the Skylab Flight Program, alterations in equipment and procedures were made for each succeeding mission to capitalize on the flight experience of the previous mission [74]. The Skylab 2 crew recommended that the personal in-flight exercise program be extended in both duration and type. To meet this recommendation, the exercise period for the Skylab 3 crew was expanded from one-half hr to 1 hr daily, and an additional exercise device was launched with the crew of Skylab 3. On Skylab 4, the duration of crew exercise was further expanded to one and a half hrs daily, and a unique treadmill device was used by the crew.

g. Space Shuttle Program

The first launch of the Space Shuttle program was in April 1981. The Space Shuttle was unique in that it was the first winged space vehicle designed to be launched from the ground and return to Earth to be reused. There have been five Shuttle orbiters, two of which were destroyed – one during launch (*Challenger*, January 1986) and the other during deorbit operations (*Columbia*, January 2003). The payload capacity of the Space Shuttle was considerable (22,700 Kg mass and 1,106 m^3 volume). Five to seven astronauts typically flew on a Space Shuttle mission. The intended use of the Space Shuttle from its inception was to support the future space station; however, for the majority of the Space Shuttle program the vehicle was used to transport

large payloads into orbit (such as the Hubble space telescope) and to conduct other low-Earth operations. The Space Shuttle cargo bay was also used to carry a laboratory, such as the Spacelab and SPACEHAB™ modules, in which human life sciences experiments were conducted.

i) Aerobic Capacity

The first studies to measure VO_2peak prior to, during, and immediately following space flight were conducted during the Space Shuttle era. Of these, only one study measured VO_2peak during space flight. Levine and co-workers [42] reported the results of peak cycle ergometer tests on six astronauts during the Spacelab Life Sciences (SLS)-1 (9 day) and SLS-2 (14 day) missions. VO_2peak measured between flight day 5 and 8 was not different than preflight VO_2peak. Interestingly, submaximal Q_c (measured on the same astronauts and same days as VO_2peak reported during the Levine study) was lower during space flight [91]. Shykoff et al. [91] speculated that either the Q_c needed to support moderate to heavy exercise is less in microgravity than on the ground or that a reduction in circulating blood volume caused by the storage of blood in the pulmonary circulation limited the increase in Q_c by reducing the SV. Reduced submaximal Q_c during space flight is difficult to reconcile with the observation that VO_2peak did not change during flight in these subjects. Additionally, Alfrey and co-workers [92] reported that plasma volume was reduced by 17% on flight day (FD) 1 and 12% on FDs 8-12 during these space flights. At this point, the decrease in Q_c without a corresponding change in VO_2peak observed cannot be explained. On landing day, subjects experienced a mean reduction in VO_2peak and Q_c of 22% and 24%, respectively, with no change in maximum HR. Levine and colleagues concluded that the post-flight reduction in maximum Q_c, and thereby VO_2peak, was due entirely to changes in SV [42]. The reduction in SV was likely due to impaired venous return caused by a decrease in plasma volume, which remained depressed on the first recovery day [42, 92]. VO_2peak recovered by approximately 50% on R+1-2 and had fully recovered to preflight levels by R+6-9 (Figure 11) [42]. Plasma volume had recovered to preflight values on R+6 [92].

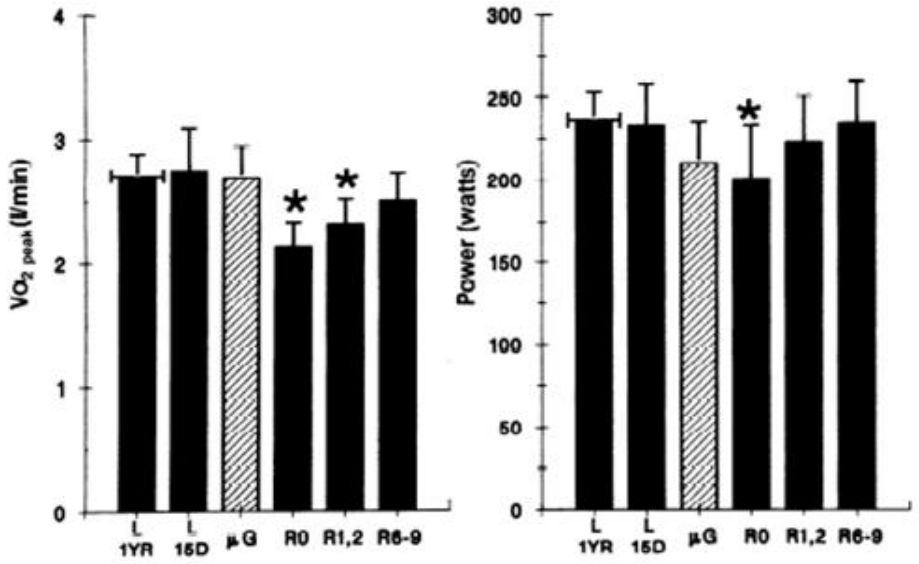

Figure 11. *VO_2max was not changed during the SLS-1 and SLS-2 flights; however it is decreased in the first 2 days following flight [42].*

Moore et al. [93] conducted a study to determine if maximal exercise performed on the last day of flight would preserve post-flight orthostatic function and aerobic capacity, as suggested by a previous bed rest study [94]. Astronauts (n=8) participating in flights ranging from 8-14 days in duration performed a peak cycle ergometer test before flight, on the last full flight day, and post-flight on R+0, R+3, and R+14. Although VO_2 was not measured during flight, the peak HR and the peak work rate performed during the in-flight tests was not different from preflight [93]. Both observations suggest that VO_2max was unchanged during flight. However, when VO_2 was measured during exercise testing on landing day, the crewmembers experienced a decline in VO_2peak ranging from 11 to 28% (mean decline 18%) [93]. Three days following flight, VO_2peak still was reduced by an average of 11% but returned to baseline values by R+14 [93]. Similar to the findings of Levine et al. [42], maximum HR did not change following flight. Thus, it is likely that reductions in Q_c and SV played a role in the decrease in VO_2peak on R+0 and R+3.

In contrast to the above studies, Trappe et al. [95] reported results consistent with aerobic deconditioning during a Space Shuttle flight. In this study, four astronauts on the 16-day STS-78 flight performed in-flight and postflight (R+4) exercise tests at a workload equivalent to 85% of preflight VO_2peak. A mean increase of 7% in exercise HR at the 85% workload was reported on FD 8, and a 9% increase was reported on FD 13, which was interpreted as a sign of aerobic deconditioning [95]. The reason for differences between these results and those reported by Levine and Moore are not readily explainable, but they may have been related to differences in the preflight fitness levels of the crewmembers participating in the studies [Trappe et al.: 3.59 $l \cdot min^{-1}$ [95], Moore et al.: 3.29 $l \cdot min^{-1}$ [93], Levine et al.: 2.76 $l \cdot min^{-1}$ [42]], exercise countermeasures and other physical testing performed during the flights, or individual differences in the response to space flight (degree of space motion sickness, medications used, etc.). Although Q_c and SV were not measured in the study reported by Trappe et al., it is possible that submaximal HRs were increased as compensatory response to a decrease in SV. On R+4 and R+8, VO_2peak was reduced by 10.3% and 5.0%, respectively [95]. This finding follows the general trend of recovery in VO_2peak observed by both Levine et al. and Moore et al.

ii) Muscle Mass, Strength & Endurance

A variety of investigations related to skeletal muscle function had been conducted during the Space Shuttle Program. One of the most comprehensive of these was a suite of investigations completed during the Extended Duration Orbiter Medical Project (EDOMP), carried out during 1989–1995 with missions of up to 16 days [14]. Studies most relevant to the risk on which this report focuses include the following: DSO 475 - Direct assessment of muscle atrophy and biochemistry before and after short space flight; DSO 477 - Evaluating concentric and eccentric skeletal muscle contractions after space flight; DSO 606 - Assessing muscle size and lipid content with magnetic resonance imaging after space flight; and DSO 617 - Evaluating functional muscle performance.

The collective specific aim of DSO 477 and DSO 617 was to evaluate functional changes in concentric and eccentric strength (peak torque) and endurance (fatigue index) of the trunk, arms, and legs of crewmembers before and after flight. LIDO® dynamometers located at the Johnson

Space Center and at both the prime and contingency landing sites were used to evaluate concentric and eccentric contractions before and after flight.

The muscle groups tested are shown in Table 3. Torque and work data were extracted from force-position curves. Peak torque, total work, and fatigue index measured in the three preflight test sessions were compared; when no differences were found between sessions, values from the three preflight sessions (L-21, L-14, L-8 days) were averaged, and this average was used to compare preflight values with those on landing day and during the postflight period (R+0, R+2, R+7-10).

Table 3. *Mean percent change on landing day from preflight mean for skeletal muscle concentric and eccentric strength of various muscle groups.*

Muscle Group	Test Mode	
	Concentric	Eccentric
Back	-23 (± 4)*	-14 (± 4)*
Abdomen	-10 (± 2)*	-8 (± 2)*
Quadriceps	-12 (± 3)*	-7 (± 3)*
Hamstrings	-6 (± 3)	-1 (± 0)
Tibialis Anterior	-8 (± 4)	-1 (± 2)
Gastroc/Soleus	1 (± 3)	2 (± 4)
Deltoids	1 (± 5)	-2 (± 2)
Pects/Lats	0 (± 5)	-6 (± 2)*
Biceps	6 (± 6)	1 (± 2)
Triceps	0 (± 2)	8 (± 6)

*Preflight >R+0 ($p < 0.05$); n=17.
Landing day (R+0) versus average of 3 preflight measures. From reference [14].

Skeletal muscle strength was defined as the peak torque generated throughout a range of motion from three consecutive voluntary contractions for flexion and extension. Eccentric contractions are actions of the muscle in which force is generated while the muscle is lengthening, as opposed to concentric actions in which the muscle is shortening (contracting) while generating force. Skeletal muscle endurance was defined as the total work generated during 25 repetitions of concentric knee exercise, as determined from the area under the torque curve for a complete exercise set. Work also was compared between the first 8 and last 8 repetitions. Endurance parameters were measured during concentric knee flexion and extension activity only. On R+0, significant decreases in concentric and eccentric strength were shown in the back and abdomen when compared to the preflight means (Table 3).

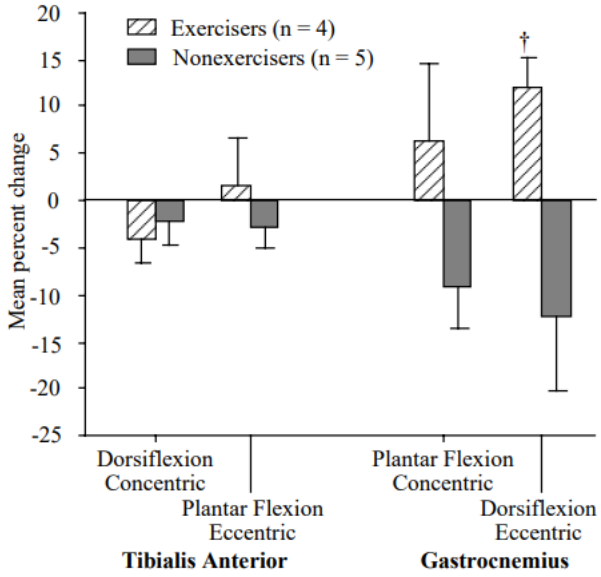

Figure 12*. Percent change in isokinetic strength in ankle extensor and flexor muscles for crewmembers who exercised during flight versus those who did not. †Preflight < R+0 ($p < 0.05$). From reference [14]*

Concentric back extension and eccentric dorsiflexion remained significantly less than preflight values on R+7. Recovery (an increase in peak torque from R+0 to R+7) was demonstrated for the eccentric abdomen and the concentric and eccentric back extensors [14].

However, the data depicted in Table 3 may be somewhat misleading as there were tremendous differences in strength between crewmembers who exercised during flight versus those who did not. For example, some crewmembers who exercised during flight actually gained isokinetic strength measured in the ankle extensor/flexor muscles (anterior

versus posterior calf muscles, that is, *m. tibialis anterior* versus the gastrocnemius/soleus complex) compared to crewmembers who did not exercise and who showed a decrease in isokinetic measured strength in these muscles (Figure 12).

Conversely, crewmembers who exercised during flight had greater losses in trunk muscle strength as measured at landing than did the non-exercising group (Figure 13). However, preflight strength in trunk flexion and extension was substantially greater in the exercising group than in the non-exercising group. Apparently, treadmill exercise did not prevent decrements in trunk strength after 9-11 days of space flight, and the investigators offered the explanation that preservation of muscle function may be limited only to those muscles that are effectively used as part of the exercise regimen.

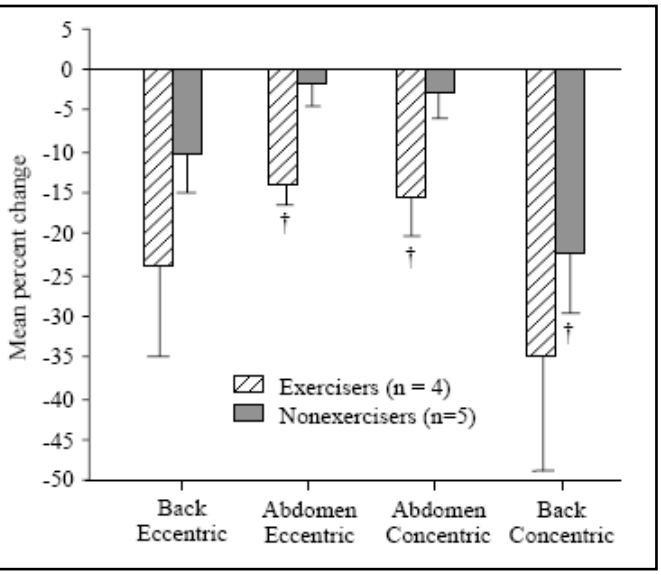

Figure 13. *Percent change in isokinetic strength in trunk muscles in crewmembers who exercised during flight versus those who did not. †Pre > R+0 ($p < 0.05$). From reference [14].*

The specific aim of DSO 475, "Direct Assessment of Muscle Atrophy Before and After Short Spaceflight," was to define the morphologic and biochemical effects of space flight on skeletal muscle fibers [14]. To obtain myofiber biochemical and morphological data from Space Shuttle crewmembers, biopsies were conducted once before flight (>L-21 days) and again on landing day (R+0). The subjects were eight crewmembers, three from a 5-day mission and five from an 11-day mission. Biopsies of the mid-portion of the *m. vastus lateralis* were obtained by means of a 6-mm biopsy needle with suction assist. Muscle fiber cross-sectional area (CSA), fiber distribution, and number of capillaries were determined for all crewmembers before and after flight.

The CSA of slow-twitch (Type I, relatively more aerobic) fibers in postflight biopsies were 17% and 11% less than in preflight biopsies for 11- and 5-day flyers, respectively [96]. Similarly, CSA of fast-twitch (Type II, relatively more anaerobic) fibers were 21% and 24% compared to preflight for 11- and 5-day flyers. Due to the extremely small sample sizes, these numbers do not reflect significant differences, but nevertheless provide evidence that space flight-induced muscle atrophy occurs at the cellular level. Interestingly, when samples were further analyzed for changes to Type II sub-types, significant CSA reductions were detected in Type IIA (−23%) and Type IIB (−36%) fibers from crewmembers involved in the 11-day flyers. The relative proportions of percent Type I and Type II fibers were different before and after the 11-day mission; the fiber distribution followed the same trend after the 5-day mission (increased Type II and decreased Type I fibers compared to preflight), but the sample size was too small to reach statistical significance. This shift is consistent with the observed reduction in the number of individual muscle fibers that expressed the Type I myosin heavy chain protein [97].

While no specific enzymatic activities involved in energy metabolism were found to be significantly different in muscle biopsy samples from returning crewmembers, the glycolytic/oxidative enzyme ratio of α-glycerophosphate dehydrogenase/succinate dehydrogenase activity was found to be increased [96], suggesting a shift resulting in decreased oxidative and increased glycolytic capacity in muscle fibers. The implication of such a shift is the

potential of reduced fatigue resistance of the muscle during work. The number of capillaries per fiber was significantly reduced after 11 days of space flight [96]. However, since the mean fiber size was also reduced, the number of capillaries per unit of CSA of skeletal muscle tissue remained the same [96]. Atrophy of both major myofiber types, with atrophy of Type II > Type I, is somewhat different from the more selective Type I myofiber atrophy observed in unloaded Sprague-Dawley and Wistar rat muscle [98-100], representing an uncommon case in which differences exist between responses of human and murine skeletal muscle.

The purpose of DSO 606, "Quantifying Skeletal Muscle Size by Magnetic Resonance Imaging (MRI)," was to non-invasively quantify changes in size, water, and lipid composition in antigravity (leg) muscles after space flight. This experiment was the first attempt to measure limb volumes before and after flight since the Apollo and Skylab programs, which used less sophisticated methods of measuring limb girths. The subjects included four Space Shuttle crewmembers from an 8-day mission. All subjects completed three preflight measurements and two postflight tests at R+1 and R+15/16. Testing involved obtaining a 1.5 Tesla MRI scan of the lower body. Multi-slice axial images of the leg were obtained to identify and locate various muscle groups. Muscle volumes for the calf, thigh, and lumbar region were measured to assess the degree of skeletal muscle atrophy. Significant reductions were observed in the anterior calf muscles (−3.9%), the gastrocnemius/soleus muscles (−6.3%), hamstrings (−8.0%), and intrinsic back muscles (−10.3%) [14]. After 2 weeks of recovery some residual atrophy persisted. These whole muscle measures along with the cellular measurements clearly established that muscle atrophy begins rapidly in the unloaded environment of space and accounts, at least in part, for the observed losses in muscle strength.

The EDOMP provided a great deal of knowledge about the effects of space flight on human physiology and specifically on alterations in skeletal muscle mass, strength, and function. Once again, losses of skeletal muscle mass, strength, and endurance were documented, in some cases despite exercise countermeasures. However, some findings were encouraging, particularly the indications that in-flight exercise does have a positive effect in countering losses in muscle strength at least in the legs (see Table 3 and Figure 8), as predicted from the results of the 84-day Skylab 4 mission when multiple modes of exercise were used including a unique "treadmill" device (see Figure 10). This unusual treadmill provided loads of sufficient magnitude to the legs in a fashion approaching resistance exercise. However, the data provided by MRI volume studies indicate that not all crewmembers, despite utilization of various exercise countermeasures, escape the loss in muscle mass that has been documented during most of the history of U.S. human space flight since Project Mercury.

In addition to the EDOMP, the Life and Microgravity Spacelab (LMS) experiments represent another hallmark Space Shuttle Program initiative to better understand the physiological adaptations to space flight. LMS was conducted aboard STS-78 and involved four crewmember subjects that participated in each of the following muscle physiology studies during their 17-day mission, described below.

Studies of muscle function and physiology. Muscle atrophy was assessed during LMS by MRI using procedures similar to those used for STS-47 [5]. Post-flight muscle volumes were significantly reduced (7–12%) in back muscles, quadriceps, gastrocnemius, soleus and gluteal muscle on landing day [101, 102]. By R+10, all changes in muscle volume had reverted to preflight levels. The observed reductions in gastrocnemius, soleus, and quadriceps muscles following the 17-day LMS mission were on average larger than those reported for the 8-day STS-47. The MRI results not only directly confirm that muscle atrophy is an early consequence of

space flight, but they also suggest that muscle atrophy continues during longer exposures to microgravity.

Whole muscle strength was measured in knee extensors and plantar flexors during LMS. The production of force by knee extensors was determined under isoinertial and isometric conditions [102]. Preflight and post-flight measurements were obtained with an instrumented leg press device that uses inertial flywheels as the resistance mode. The device could also be locked in place at a 90-degree knee angle for the measurement of maximal isometric force. Consistent with the reported reduction in quadriceps cross-sectional area, knee extensor (leg press) strength was reduced post-flight (R+1). Maximal isometric force was reduced by 10.2%; whereas concentric and eccentric strength were reduced 8.7% and 11.5%, respectively [102].

In separate experiments involving the same astronaut subjects, calf muscle performance was assessed before, during, and after STS-78 with a torque-velocity dynamometer (TVD) [103]. The TVD was a mission-specific piece of hardware that measured ankle plantar flexion and dorsiflexion strength under isometric or isokinetic (fixed angular velocity) conditions. Angle-specific tests for isometric strength (80, 90, 100 degrees), isokinetic strength at speeds from 30-360 degrees/seconds, and isokinetic endurance were measured before, during and post-flight. In-flight measures were conducted on flight day (FD)2/3, FD8/9, and FD12/13. Postflight measures were assessed on R+2 and R+8. Muscle strength values were reported to be ~50% lower during the first two in-flight time points, but the charges were attributed to issues with the system that secured the TVD in place [103]. The TVD was reported to be “lifting and floating” during testing. The issue was resolved prior to FD12/13 testing at which time differences in torque generation compared to preflight were observed. Likewise, post-flight measures were not significantly different than from preflight values. The authors of the investigation have suggested that the lack of change during 17 days of space flight may have been due to the nature in which the testing was conducted. That is, the in-flight testing may have served as an unexpected, yet effective, exercise countermeasure to protect the calf muscle from strength loss. The three in-flight calf muscle test sessions during STS-78 involved making ~525 calf muscle contractions on the TVD [103], half of the of which were made at 80% to 100% of each individual’s maximal values [103, 104]. By contrast, the same LMS crew displayed significant deficits in both size and strength of the quadriceps [102], a muscle group that was not tested during flight. This suggests that high-intensity muscle contractions, which are performed less than daily, may protect muscle strength during missions of up to 17 days.

Loss of skeletal muscle strength is a consequence not only of reduced muscle size but of decreased neural drive and myocellular damage. Studies were performed on the calf muscles (contralateral leg to studies described above) before flight, during flight (four time points), and after flight to separate the causal effects of muscle atrophy from reduced neuromuscular recruitment [105] to address this question. Surface electrodes were placed over the subjects’ gastrocnemius and soleus, and a percutaneous electrical muscle stimulator (PEMS) unit was used to directly cause forced whole-muscle contractions independent of any voluntary input provided by the crewmember. No measurable losses in electrically-evoked calf muscle performance were observed [105]. However, post-flight (R+8) reductions in force production were observed. Given the lack of change during late in-flight testing (FD16), it was suggested that alteration may have been due to muscle damage from gravitational reloading of the muscles during normal ambulation. This notion was supported by MRI analyses. MRI transverse relaxation time (T2) of skeletal muscle, an indicator of increased tissue fluid volume and a marker of myocellular damage (inflammation/edema), was elevated at R+2 and stayed elevated at R+10 in these crewmembers.

Studies of muscle morphology and cellular function. Muscle biopsy samples were obtained from the 4 LMS crewmembers who participated in the whole-muscle size and function testing [103, 106-109]. Biopsies were taken from the gastrocnemius and soleus muscles before flight and again within three hrs of landing. Functional analyses of single muscle fibers provide the most direct evidence of space-flight-induced changes in the function of the muscle mechanics without the influence of factors such as changes in neuromuscular recruitment pattern or differences in volitional effort. Using individual muscle fibers, any observed alterations in mechanics can be attributed to alteration in the myofiber itself. Individual muscle fibers from LMS crew were isolated and mounted between a force transducer and a servomotor for analyses. Space flight produced a small decrease (−6%) on type I single-fiber peak calcium-activated force production (P_o) in samples from the gastrocnemius [109]. However, no difference was observed when these measurements were corrected for muscle fiber cross-sectional areas. No mean differences were found in P_o or fiber cross-sectional area for fibers that either expressed type IIa MHC or co-expressed both type IIa and IIx MHC. While mean differences in fiber mechanics were not observed in subjects as a group, significant changes occurred within individual subjects when subject-by flight analyses were conducted (each subject had a cohort of fibers that were analyzed). In one subject, P_o and cross-sectional area in type IIa fibers were reduced by 19% and 12%, respectively. In yet another, P_o was reduced by 23% in type I fibers and 15% in type IIa fibers, with reductions in fiber cross-sectional area of 7% for type I and 12% for type IIa [109]. The investigators point out that the variability in space flight response seems to result, at least in part, from initial fiber size. Fibers with the greatest reduction in size and P_o tended to come from the crewmembers who had larger preflight fibers.

In the soleus muscle, a calf muscle adjacent to the gastrocnemius but one that is more slow twitch and oxidative in nature, 91% of muscle fibers expressed only type I MHC before flight [108], After space flight, the number of type I fibers dropped to 79%. Space flight also resulted in a 21% in mean P_o. This decline in Ca^2-activated peak force was paralleled by a 15% decrease in fiber CSA [108], which indicates that muscle atrophy accounted for most of the loss of function, although a 4% residual loss of P_o remained when P_o was normalized by individual fiber CSA.

Skeletal muscle power is generally viewed as a functional measure of muscle performance because, like most physical tasks that require high levels of exertion, peak values actually occur at submaximal loads. The power of single fibers was measured in a manner similar to the P_o measurements; but instead of the measures being isometric, they are made with isotonic load clamps. No significant main effect of space flight was found on muscle power for single fibers from either the gastrocnemius [109] or the soleus [108] muscles. Despite some variability among crewmembers in the effect of space flight on P_o in various muscle fiber types, the overall trend showed that increases in maximal shortening velocity (V_o), which are attributed to decreased thin filament density based on observations from electron microscopy [106, 107], compensate for the loss of P_o to maintain muscle power at the cellular level.

Skeletal muscle is a highly metabolic tissue. As is true for muscle size, the intensity and the volume of physical activity are also major determinants in the readily adaptable bioenergetic capacity and makeup of the muscle. Portions of the biopsy specimens from the gastrocnemius and soleus were used to perform biochemical analyses of oxidative and glycolytic enzymes. Despite some evidence of a metabolic shift towards glycolysis-derived energy sources in biopsy samples after the 11-day STS-32 mission [110], no differences were detected in citrate synthase, phosphorylase, or β-hydroxyacyl-CoA dehydrogenase in samples after the 17-day LMS mission [103]. Accordingly, no post-flight changes were observed in muscle glycogen content. Therefore, while space flight appears to promote a slow-to-fast shift in MHC, there does not appear to be a similar systemic metabolic shift.

iii) Exercise Countermeasures

Investigations related to exercise capacity and the preservation of the cardiovascular responses to exercise were conducted by NASA during the Extended Duration Orbiter Medical Project (EDOMP) from 1989 to 1995. These studies were 1) designed to be relevant to space flight operations, 2) required to be related to performance of the crewmembers during entry, landing, or egress from the Space Shuttle, and 3) conducted as NASA Detailed Supplemental Objectives (DSOs) [14]. DSO studies are limited in the amount of hardware stowage that can be used to support the studies during flight; therefore, the majority of these involved pre- and post-flight comparisons. In addition, fairly early in EDOMP, NASA's Committee for the Protection of Human Subjects limited the intensity of exercise investigations during and immediately following space flight to levels of no greater than 85% of preflight VO_2peak. The authors of this report are not aware of any cardiovascular anomaly that occurred either during or following flight that precipitated this exercise limitation. In any event, this restriction is the reason for the limitation of exercise intensity of the subjects of Trappe et al. and subsequent investigations [111]. Despite the above listed limitations, studies conducted during the EDOMP era produced findings related to the space flight-induced decrease in aerobic capacity. One study examined the effects of continuous versus low-level interval exercise on post-flight aerobic capacity [112]. During flight the astronauts (n=17) performed either continuous exercise or interval exercise on a small passive treadmill or served as controls (Figure 14). HR was used by the exercising crewmembers to regulate exercise intensity. Treadmill testing to measure VO_2peak was performed before and 2 days following flight. VO_2peak was maintained in both exercise groups, while the control subjects experienced a 9.5% loss [112]. Although this study did not measure VO_2peak immediately following flight, it demonstrated that VO_2peak following flight could be altered by in-flight training.

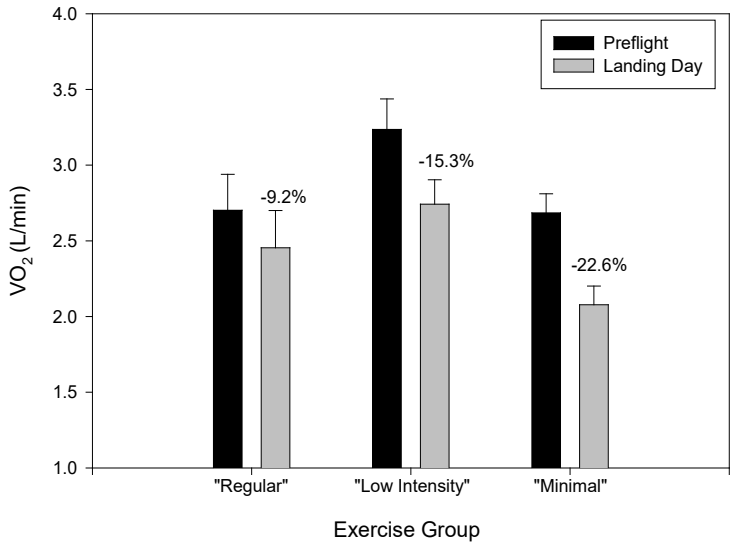

Figure 14. *Oxygen consumption achieved at 85% age-predicted maximum HR pre- and postflight in crewmembers (n=35) who participated in different amounts of in-flight exercise. "Regular" (n=11) - Exercised > 3x/week, HR > 70% age-predicted, > 20 min/session. "Low Intensity" (n=10) - Exercised > 3x/week, HR < 70% age-predicted, > 20 min/session. "Minimal" (n=14) - Exercised < 3x/week, HR and min/session variable. Redrawn from Greenisen, et al [14].*

Another study conducted during EDOMP was designed to monitor aerobic exercise performed during flight and the influence of this exercise on the HR and VO_2 responses to exercise testing following flight [113]. Astronauts (n=35) performed incremental upright cycle ergometer exercise tests (50 W for 3 min, followed by 50 W increases every 3 min) with VO_2 and HR measurements prior to flight (L-10) and on landing day (R+0). These tests were terminated at the work stage that elicited 85% of each participant's age-predicted maximum HR; VO_2peak was not measured. Exercise countermeasures for use during flight were not prescribed, but each astronaut wore an HR monitor that recorded both the HR and duration of their exercise sessions. Most exercise sessions were completed on the Space Shuttle cycle ergometer (a treadmill was only available on one mission). The major finding of the study was that astronauts who performed regular aerobic exercise during flight demonstrated a smaller elevation in HR at the termination workload than the astronauts who exercised less frequently or at a lower intensity (Figure 14). Regular aerobic exercise was defined as three or more sessions per week, each session lasting at least 20 min and at an intensity that elicited a HR of >70% of their age-predicted maximum HR [113]. Q_c was not measured in these subjects, but the relative tachycardia experienced by the crewmembers on landing day is consistent with a compensation for lowered SV. Though speculative, it is possible that plasma volume was better maintained in the "regular exercise" subjects. Lee and co-workers who reported on the R+0 stand test findings of these subjects [1] observed a greater HR response and reduced pulse pressure (often used as an index of SV) during standing in the "minimal" exercise subjects. Thus it appears that, at least for Shuttle duration flights, a decline in VO_2peak immediately following flight may be partially attenuated by exercise conducted during flight.

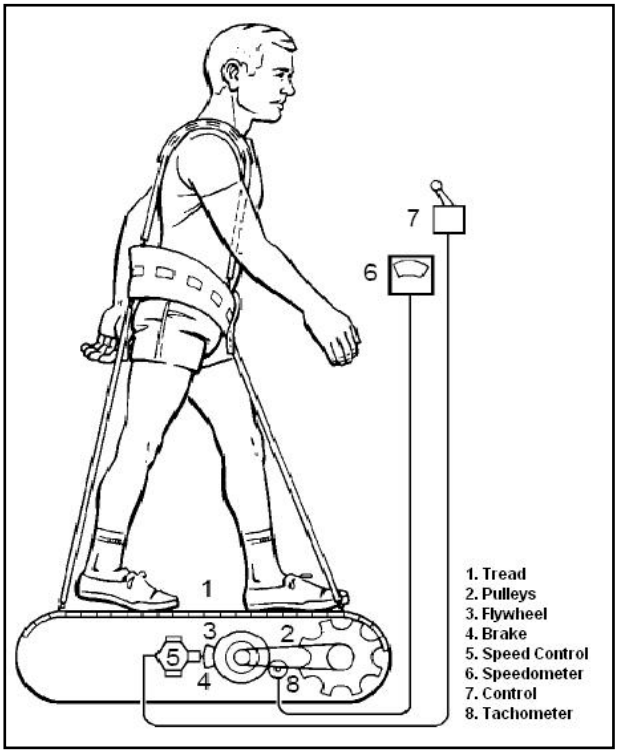

Figure 15. *First-generation or original Space Shuttle passive treadmill. From reference [14].*

During the DSO 477 and DSO 617 tests, subjects in this study exercised during flight for various durations, intensities, and numbers of days on the original Shuttle treadmill (Figure 15) (as opposed to the EDO treadmill, which flew on later Shuttle missions and was the basis for the ISS treadmill) as part of separate in-flight investigations. Exercise protocols included continuous and interval training, with prescriptions varying from 60% to 85% of preflight $VO_{2\text{-max}}$ as estimated from heart rate (HR). Some subjects had difficulty in achieving or maintaining their target HR during flight. The speed of this passive treadmill was controlled at seven braking levels by a rapid-onset centrifugal brake (see Figure 15). A harness and bungee/tether system were used to simulate body weight by providing forces equivalent to an approximate 1-g body mass. Subjects on this non-motorized treadmill were required to walk and run at a positive percentage grade to overcome mechanical friction. Study participants were familiarized with the LIDO® test protocol and procedures about 30 days before launch (L-30), after which six test sessions were conducted. Three sessions were completed before launch (L-21, L-14, and L-8 days) and three after landing (R+0, R+2, and R+7 to R+10 days).

h. Relevant Data from the Shuttle-Mir and NASA-Mir Programs

During the seven NASA-Mir flights, seven U.S. astronauts trained and flew jointly with 12 Russian cosmonauts over a total period of 977 days of space flight (the average stay was 140 days), which occurred during the period from March 1995 to June 1998. The major contribution of the joint U.S./Russian effort on the Mir space station relevant to the current risk topic was the first use of MRI to investigate volume changes in the skeletal muscles of astronauts and cosmonauts exposed to long-duration space flight. This began with the first joint mission, Mir-18, and continued until the final Mir-25 mission. The data indicated that loss of muscle volume, particularly in the legs and back, was greater during long-duration space flight than with short-duration space flight, but not as great as the data from short-duration flight might have predicted [114]. A comparison between volume losses in the selected muscle groups in short-duration space flight on the Space Shuttle, long-duration (119 days) bed rest, and a (115 d) Shuttle-Mir mission demonstrates the relative time course of the losses (Figure 16).

There is a correlation between long-duration bed rest and space flight of similar duration, except that the losses in the back muscles are less with bed rest. This likely reflects use of these muscles during bed rest to adjust body position and to reduce the potential for vascular compression and tissue injury. The back muscles are used less frequently during space flight because they do not have to support the upright body against Earth gravity and are not used with as much force compared to bed rest where subjects use back muscles to make positional adjustments of the body.

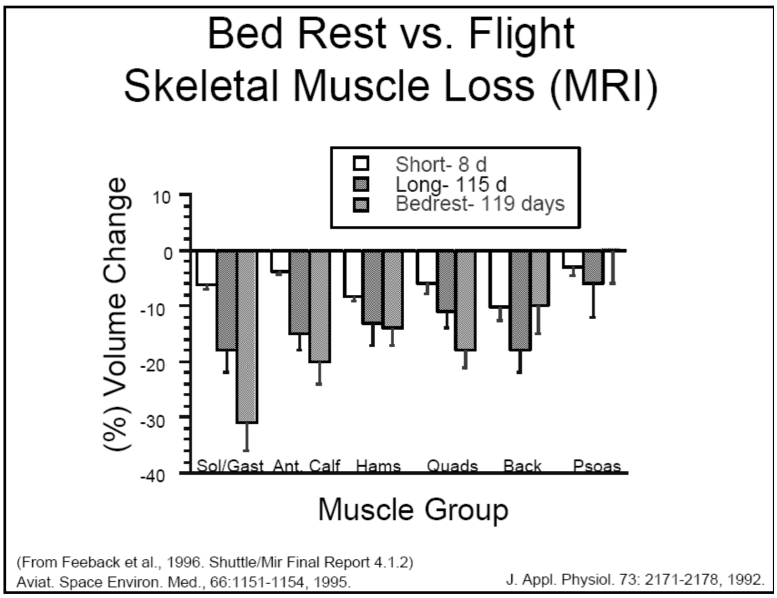

Figure 16. *Percent change in selected muscle groups during short (8 d; n = 8) and long (115 d; n = 3) spaceflight (Mir 18) compared to long-duration bed rest (119 d). Data from references [5, 6] and the Shuttle/Mir Final Report.*

i. International Space Station (ISS)

The ISS is a low-Earth orbiting research facility. ISS assembly in space was initiated in 1998, and a manned presence on board ISS has continued since November 2000. The crews of the ISS have been comprised of U.S. (NASA), Russian (Roscosmos), European Space Agency (ESA), Canadian Space Agency (CSA), and Japanese Exploration Agency (JAXA) astronauts. The crew size for ISS Expeditions has varied between two and six long-duration occupants. The U.S. Space Shuttle carried astronauts to and from the ISS until its retirement in 2011. Between 2011 and spring of 2020, the Russian Soyuz vehicle was the sole method of supplies and crewmembers' delivery to and from ISS. Following the successful completion SpaceX's Demo-2 mission in spring 2020, SpaceX's Falcon 9 rocket and Crew Dragon Spacecraft have been ferrying NASA astronauts and International Partners to the ISS as a part of the Commercial Crew

Program. As of 2021, there have been 64 ISS long-duration expeditions with average mission lengths of 4-6 months (Figure 17).

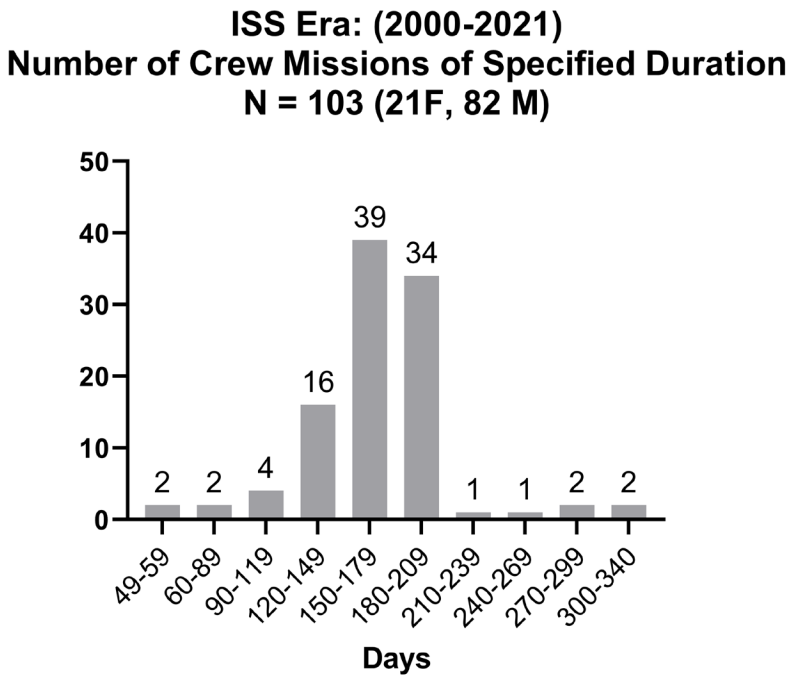

Figure 17. *Source: U.S. Space Program Flight Durations, Worksheet: ISS. Current through Exp 64/SpX-2 (2021). Includes International Partners, with the exception of a handful of ESA crewmembers who had direct return to Europe after landing.*

i) Aerobic Capacity

Aerobic capacity research has been conducted on the ISS from its inception. The goal of any countermeasure to space flight exposure is to preserve the capability of the crewmembers to perform daily tasks and EVA or emergency egress tasks that may require high levels of work for extended periods of time or in repeated bouts. Regarding routine tasks conducted on board the ISS, EVAs typically elicit an average metabolic cost of ~ 200 kcal·hr^{-1} (~0.7 L O_2 ·min^{-1}) and have ranged up to 500 kcal·hr^{-1} (~1.7 L O_2 ·min^{-1}), which equates to approximately 50% of the typical astronauts VO_2peak. However, EVAs can become prolonged and aerobically challenging because activity can last for up to 8 hrs, and the work is predominantly upper body in nature (VO_2peak measured during upper body exercise is approximately 70% of that measured during lower body exercise). The metabolic cost of performing an emergency egress task in the NASA Launch and Entry suit has been reported as ranging from 2.0-2.7 Liters O_2 ·min^{-1}, depending upon the amount of G-suit pressurization employed [70]. This has been reported to be about 70% of a person's VO_2peak [115]. With regard to EVA on the lunar surface during the Apollo era, several EVAs reportedly were slowed by request of the monitoring flight surgeons as heart rates during the activities reached 150-160 beats/min [71]. Until the mission scenarios are defined for future EVA work, it is difficult to predict precisely what VO_2peak will be required to successfully complete all tasks. However, it is likely that future exploration tasks will be more demanding and will need to be performed with more autonomy as real-time communication with the ground may not be possible. Consequently, the importance of maintaining VO_2peak will not diminish as space exploration moves from ISS to planetary exploration.

Aerobic fitness has been evaluated during and following space flight starting as early as the Gemini Project. This early evaluation on aerobic capacity was based on the linear relationship between VO_2 and HR response to submaximal exercise. Submaximal tests extrapolated submaximal VO_2 to maximal HR and estimated VO_2peak. However, these were known to not be accurate measures of VO_2peak.

Several studies have measured VO_2peak during flight using metabolic gas analysis systems. Levine et al. [42] showed VO_2peak was unchanged during short-duration space flight. Trappe et al. [9] compared short-duration 17 day bed rest to space flight and reported reduced (7-10%) aerobic capacity upon return to Earth on R+3 (Figure 18). This was in agreement with Moore et al. [47] that showed that VO_2peak is severely impaired early in-flight (the first 2 weeks) and gradually trends upward during flight but does not reach preflight levels.

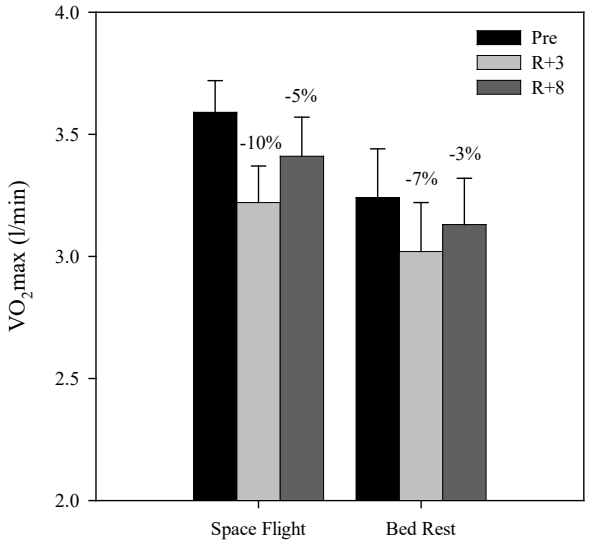

Figure 18. *VO_2peak before, 3 days after (R+3), and 8 days after (R+8) 17 days in space or bed rest [9].*

Moore et al. [47] measured VO_2peak in long-duration ISS astronauts (9 male, 5 female) ~90 days before flight, 15 days after launch (flight day, FD15), every ~30 days in-flight, and 1, 10, and 30 days after landing (Recovery, R+1, R+10, and R+30). The mission durations ranged from 91 to 192 days and occurred during Expeditions 19-33. The peak cycle test protocol consisted of the first three stages of the submaximal protocol (3, 5-min stages set at 25%, 50%, and 75% of preflight VO_2peak) followed by 1-min stages of 25 W increments to volitional fatigue. All tests were performed at preflight and postflight in the upright posture on an electronically braked cycle ergometer. All in-flight tests were performed on the CEVIS. Metabolic gas analysis measurements were made to determine VO_2peak using the Portable Pulmonary Function System (PPFS). Technical details on the PPFS system can found in Clemensen et al. [116]. The main findings of this study were that VO_2peak decreased by 17%, and peak workload decreased by 24% from preflight to FD14. VO_2peak and peak workload then gradually increased during flight but never returned to preflight levels (Figure 19). VO_2peak was lower on R+1 (~15%) and R+10 than preflight but recovered by R+30. Peak HR was not different from preflight at any time during or following flight. The individual data showed that most, but not all, astronauts with higher initial fitness levels experienced greater decreases from preflight to the first in-flight VO_2peak test; however, they typically remained at higher levels than the lower fit astronauts. The relationship between pre- to in-flight VO_2peak was significantly correlated ($R^2 = 0.59$; $p = 0.006$) [47].

Although most astronauts experienced a decline in VO_2peak during the mission, four of the astronauts with average to above average fitness levels preflight maintained their VO_2peak within 3% of preflight values during space flight, providing important evidence that aerobic deconditioning is not an inevitable consequence of long-duration space flight. Notably, these four astronauts exercised on the CEVIS at a higher percentage of peak HR (79±6% vs. 68±20%, mean ±SD) and spent a greater percentage of their time exercising above 70% of peak HR (76±30% vs. 63±32%, mean±SD) compared to those who experienced a decrease in VO_2peak in this cohort [47]. These are the first data to show high intensity exercise is required for astronauts to maintain VO_2peak during space flight. A larger data set, however, is required before recommendations can be made towards redefining exercise prescriptions and minimal intensity requirements.

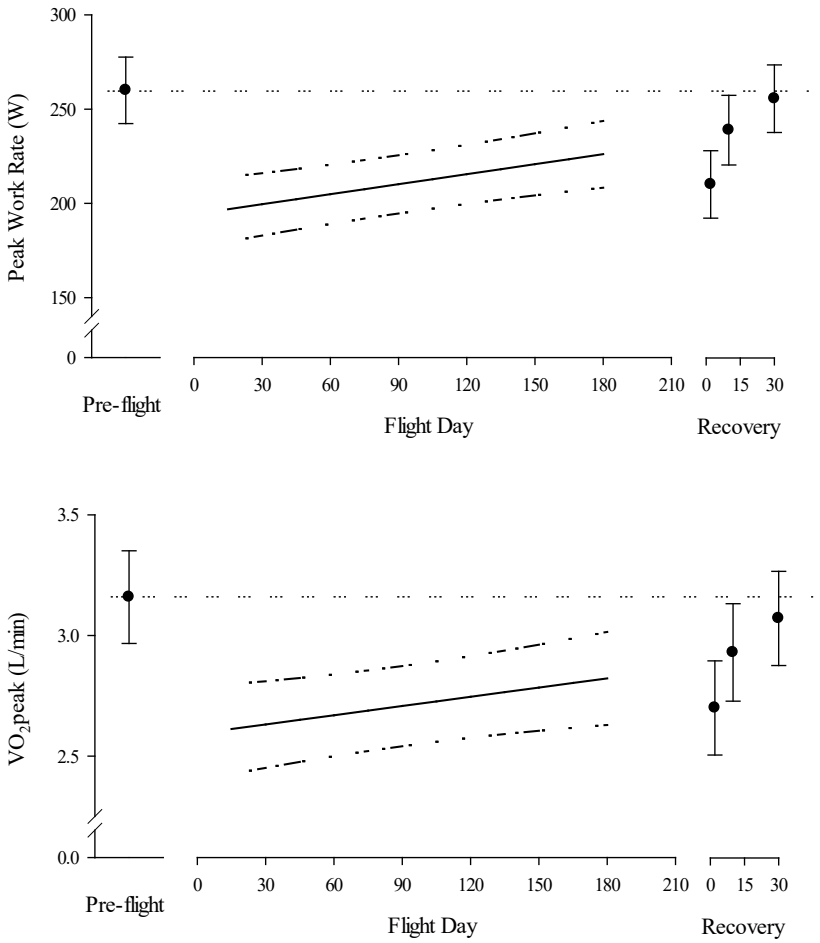

Figure 19. *Effect of long-duration space flight on peak work rate (top) and absolute VO_2peak following ISS missions. The horizontal broken line is a reference drawn at the preflight mean baseline. The solid line ± dashes during flight are the mixed-modeled linear regression mean responses ± 95% CI. [47].*

These observations on the understanding the importance of exercise intensity were investigated by English et al. [52]. This recent in-flight exercise prescription study (i.e., the SPRINT Study) has provided more insight towards exercise prescription recommendations to maintain VO_2peak during space flight [52]. This study evaluated the effectiveness of a new training

prescription, where ISS crewmembers performed a high intensity/lower volume integrated resistance (3 d/wk) and aerobic (interval and continuous workouts, each 3 d/wk in alternating fashion) exercise program (SPRINT). The control group (CON) performed the standard ISS exercise countermeasure program consisting of daily resistance and aerobic exercise during long-duration space flight. After space flight, VO_2peak (−CON: -7%, SPRINT: -10%) had comparable decrease in both groups (preflight to postflight: CON, 3.36±0.19 to 3.12±0.19 L/min versus SPRINT 3.22±0.23 to 2.91±0.23 L/min) [52]. However, it is important to address the limitations, which included low sample size with inadequate power, bias sample selection of crew that chose to participate, and those whom did not want to make changes in their exercise prescriptions. Additionally, it was noted that CON subjects had large variability in their prescription of exercise. Moreover, SPRINT group tested on R+1 while the CON group tested on R+3. Plasma volume is known to have large effects early upon return that affects VO_2peak test (see section I Factors that Affect Aerobic Capacity for detailed description). However, the importance of this study shows that reduced time of exercise may be effective only if intensity of exercise is countered within the prescription of exercise. The SPRINT high intensity/lower volume training may be a time effective program of space flight exercise countermeasure prescriptions.

For the ISS astronauts, the early in-flight decline of VO_2peak is likely due to several factors including time without exercise in the several days, up to a week, following docking with the ISS. Additionally, cephalic fluid shifts contribute to a decrease in blood volume, primarily the plasma component, potentially resulting in decreased muscle perfusion pressure as well as initiating some degree of central cardiovascular deconditioning [117]. The trend for improved VO_2peak observed during flight may be due to physiological normalization to the space flight environment, as well as the cardiac, blood volume, and peripheral muscle training adaptations expected in individuals who perform regular aerobic exercise.

ii) *Aerobic Capacity Means Pre- to Postflight*

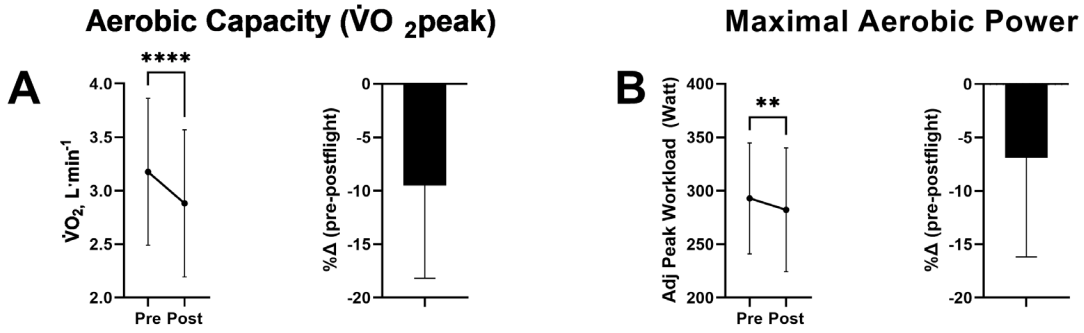

Figure 20. *Indices of aerobic fitness. Aerobic capacity (A), and maximal aerobic power (B) are reduced pre- to postflight while exercise economy at VO_2peak is similar. ISS dataset pre- to postflight (L-3/1mo to R+3d), mission duration 185±48 days, n=47 (male 36/ female 11), Mean ± SD, ** = P<0.01, **** = P<0.0001*

Studies that have measured VO_2peak upon landing (conducted on short-duration Space Shuttle astronauts and on long-duration ISS astronauts) have consistently demonstrated that VO_2peak is significantly lower on landing day than it is before flight [42, 47, 93]. VO_2peak appears to recover within 6-9 days following landing after short-duration missions [42, 93], but requires a longer time to recover after long-duration missions [47]. After long-duration, the postflight results

show a significant decrease in VO_2peak (~10%) immediately upon landing with a return to preflight levels by R+30 and is consistent with previous space flight investigations [42, 118]. However, VO_2peak was still below preflight levels on R+10 in the astronauts [47], indicating that the recovery time may be longer after 4–6 month ISS missions, compared to short-duration Space Shuttle missions. Here we report NASA JSC preliminary unpublished data on the absolute and relative changes pre- to postflight in a larger sample (n=47) and find that aerobic capacity and maximal aerobic power are reduced about 9–7% after long-duration ISS missions (Figure 20). However the large variability of the crew population is concerning.

iii) *Aerobic Capacity Pre- to Postflight Individual Variability*

Understanding the exercise response variability for human health and performance is of utmost importance in precision exercise medicine and is key to understanding the appropriate level of exercise and hardware countermeasures to use for maintaining fitness of the astronaut crew [119-121]. The individual response to exercise is a major gap in knowledge at NASA. It is reported that not every person responds positively to endurance training on Earth (1g). Notably, about ~20% of individuals do not respond to exercise training. These individuals are characterized as non-responders whose individual responses may be due to differences in genetic profiles, genetics being the most potential biological contributor to variability of the exercise response is genetics (~50% variability is explained) [122]. Additionally, methodological factors contribute to this variability such as instrumentation calibration, validity, repeatability, and operator error. However, it is important to note that age, baseline cardiorespiratory fitness, sex, methods of exercise prescription (i.e., absolute HR, VO_2, W, speed, percent of maximal capacity of either VO_2, W, or HR), frequency, intensity, time, volume, and type of exercise (i.e., resistance, aerobic cycling/treadmill) are some important factors that contribute to the individual variability response [119, 122]. Notably, Ross et al. [123] reported that low volume/low intensity exercise (300 kcal per session at 50% of VO_2peak) was not sufficient to eliminate non-responders (39% of group) from exercise training, while high volume/low intensity (360 and 600 kcal per session at 50% of VO_2peak) or high volume/high intensity (360 to 500 kcal per session at 75% of VO_2peak) was adequate to reduce or eliminate non-responders (18% and 0% respectively) [123]. These results suggest an appropriate volume and intensity level of exercise for all individuals is possible.

The characterization of a responder from exercise training is an individual who does respond positively to exercise training. This means that their fitness (aerobic capacity or strength) levels improve after an exercise intervention. A non-responder is characterized as an individual who shows no improvements after the same exercise intervention. In the context of spaceflight, the primary objective to exercise countermeasures is to maintain fitness over the course of spaceflight and upon return to Earth. Therefore, for this evidence report, the definition of a responder is a crewmember that maintains fitness from pre- to postflight. The definition of non-responder is a crewmember that does not maintain fitness from pre- to postflight outside of the measurement error. The major assumption in this characterization is that both responders and non-responders are completing the same nominal ISS exercise prescription.

Figure 21 provides preliminary ISS data on the characterization of responders and non-responders to in-flight exercise prescription (same sample of Figure 20). Instrument variation has been reported to be with ±3% for within-subject variations for VO_2, VCO_2, and VE and ~2% for inter-unit instrument error for a standard gas analysis system [124]. Our preliminary data suggest that only 30% of crew population are responding well to the exercise prescription in-flight and maintaining fitness within the measurement error. Of that 30%, only 4% of crew return with improved aerobic capacity. Overall, 53% of crew lose greater than 10% of their aerobic capacity.

Moreover, of the entire crew population within this dataset,15% of crew lose greater than 20% of their preflight aerobic capacity upon return to Earth.

Understanding and improving the dose of exercise is necessary to make sure all crew return safely while maintaining health and performance. Moreover, future long-duration missions (i.e., Artemis) to the Lunar and Martian surface will require adequate countermeasures to maintain fitness to complete critical mission tasks (e.g., return to Earth and planetary egress and space walking with suits) with reduced ISS hardware capabilities.

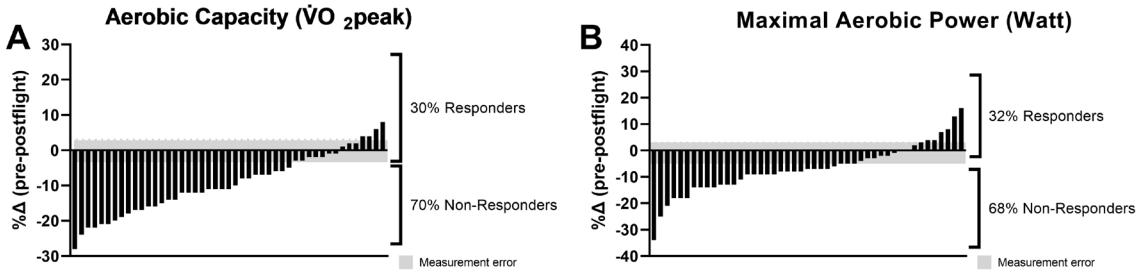

Figure 21. *Individual variability on indices of aerobic fitness. ~70% of crew do not respond to the current exercise interventions for maintaining aerobic capacity (A) and maximal aerobic power (B). A responder is defined as maintaining preflight fitness within measurement error (gray box ±3%). ISS dataset pre- to postflight (L-3/1mo to R+3d), mission duration 185±48 days, n=47 (male 36/ female 11)*

iv) Muscle Mass, Strength, & Endurance

Two major research study complements addressing the *Risk of Impaired Performance Due to Reduced Muscle Mass, Strength, and Endurance* were conducted during the early phase of the ISS exercise that assessed the countermeasures' capability. Subjects had access to the CEVIS cycle ergometer, the TVIS treadmill, and importantly, the interim Resistive Exercise Device (iRED). iRED is an elastomer-based piece of resistance exercise hardware. This device was limited to a 300-lb maximum load. By comparison, the currently available Advanced Resistance Exercise Device (ARED) has a 600-lb load capability. One investigation during the "iRED era" involved four ISS astronauts with mission durations of 161–194 days [57], and the other studied 10 astronauts and cosmonauts whose mission durations spanned a very similar 161–192 days in space [125-127]. Each of these studies investigated changes to muscle size and strength with one focusing on a larger array of muscle groups and the other performing a diverse set of whole muscle, cellular. And biochemical measures on the postural muscles of the calf.

Initial post-landing MRI data for both studies were conducted on a relatively similar timeline (5±1 and 4±1 days). Calf muscles were found to undergo the greater decrements than thigh muscles (10-18% vs. 4–7% loss, respectively) [57]. Both studies reported the greatest loss in the soleus muscle (15-19% loss), with loss of a lesser extent in the gastrocnemius muscle (10% loss) [57, 127]. Approximately half of the loss of muscle mass still existed up to 2 weeks following return to Earth [127]. Although these MRI results highlight a clear need for improved countermeasures hardware and/or strategy, they also evidence an incremental improvement in the countermeasures targeted to mitigating muscle loss when compared to the more dramatic reductions observed during Shuttle-Mir missions [114]. Muscle strength measurements in ISS crewmembers were not measured until approximately a week following landing. Nonetheless, strength losses accompanied muscle atrophy in both upper [57] and lower leg muscles [57, 127].

Isokinetic strength measures in knee extensor muscles revealed a 10% loss [57]; whereas calf muscle strength was reduced by 24% [57, 127], again demonstrating that the calf muscles are most susceptible to space-flight-induced decrements. The drop in torque production on the calf muscles were observable across the entire range of speeds used from 0–300 degrees/second [127]. This reduction in calf muscle performance, taken initially one week post landing, persisted until at least two weeks after return despite a partial restoration in muscle volume [127]. Taken together, the results suggest that impairments in muscle strength are likely perturbed by muscle damage and/or soreness brought on my gravitational reloading of the muscles.

Various structural and functional analyses were performed on muscle biopsy samples from the gastrocnemius and soleus muscle from nine ISS crewmembers [126, 127]. Mirroring what was observed on the whole muscle level, individual muscle fiber analyses also revealed muscle atrophy at the cellular level [126]. CSA were determined in individual muscle fibers which were set at a standardized sarcomere length. The slow type I muscle fibers were reduced by 24% and 33% in the gastrocnemius and soleus muscles, respectively. Fast type II fibers (of all sub-types, excluding hybrids) were also reduced in the soleus muscle (29%), but unchanged in gastrocnemius. Measures of muscle fiber mechanics clearly demonstrated decrements of function at the cellular level [126]. Peak calcium activated force, maximal shortening velocity, and peak power were all markedly reduced in post-flight samples taken from gastrocnemius and soleus muscles with the most dramatic change being a 45% loss of power production in type I fibers in the soleus muscles. This is in stark contrast to results from short-duration Space Shuttle flights where increases in maximal shortening velocity were able to compensate for reduced force production in order to maintain peak power levels. Power was also reduced in type II fibers, with reductions to maximal shortening velocity and peak force being contributing factors for fibers from gastrocnemius and soleus muscle, respectively [126, 127]. Mirroring what was observed on the whole muscle level, individual muscle fiber analyses also revealed muscle atrophy at the cellular level [126].

In both gastrocnemius and soleus muscle, a clear shift in the contractile machinery was observed with a slower-to-faster phenotype reported [127]. This can be observed from MHC protein expression from the individual fibers that were analyzed for contractile properties. Both gastrocnemius and soleus muscle exhibited reductions in the amount of fibers expressing type I MHC. This corresponded with increases in the percentages of type Iia fibers and Type I/Iia hybrid fibers from gastrocnemius muscle. A similar pattern occurred in the soleus muscle though increases were primarily observed in the various hybrid fibers distributed in a manner such that significant changes were only detected in hybrid fibers grouped together.

Limitations in the availability and accuracy of iRED loading data prevented investigators from making meaningful analyses of the relationships between resistance training loads and muscle adaptions during these ISS missions. However, a number of observations were made regarding treadmill running and changes in the calf muscles [127]. Treadmill use ranged from less than 50 min a week to greater than 300 min per week. Subjects who ran on the treadmill the most preserved muscle strength better than those who ran less. When total aerobic exercise (TVIS treadmill + CEVIS bicycle ergometer) was compared to changes in muscle volume, this correlation was lost. Data demonstrating that foot forces are much higher during treadmill running versus cycling aboard ISS [128] supports the argument that higher forces are vital for protecting against muscle atrophy during space flight. Results for treadmill use were not restricted to in vivo whole muscle observations. Subjects who used the TVIS treadmill more than 200 min per week generally fared better than those who ran less than 100 min per week in terms of single fiber cross-sectional area, peak force, and power [126]. An evaluation of ground reaction force during a parabolic flight reported that peak force and total work were significantly less, by 42-46% and

33-37%, comparing 1g to 0g for the iRED [129]. This data suggest that crew must exercise at greater external resistive forces in-flight compared to on Earth.

In addition to muscle mass and the function of the cellular contractile proteins, changes to the molecular mechanisms that control energy metabolism also have the potential to negatively affect human performance following exposure to long-duration space flight. Activities of a battery of oxidative and glycolytic enzymes were therefore measured in crewmembers before and after ISS missions [125]. By and large, the observed space flight effects on metabolic enzymes in skeletal muscle were minimal. No changes in activities of citrate synthase, β-hydroxyacyl-CoA, lactate dehydrogenase, or phosphofructokinase were observed in calf muscles following 6 months aboard ISS. Rather, space flight and exercise countermeasures play a more limited role in select adaptions to metabolic enzymes in calf skeletal muscles. For example, the mitochondrial enzyme cytochrome oxidase was reduced in space flight by 35% in type I fibers in the soleus muscle for all crew studied. However, this was entirely accounted for by those in the low treadmill use group (less than 100 min/wk) where a 59% reduction occurred. Activity levels in the high treadmill use

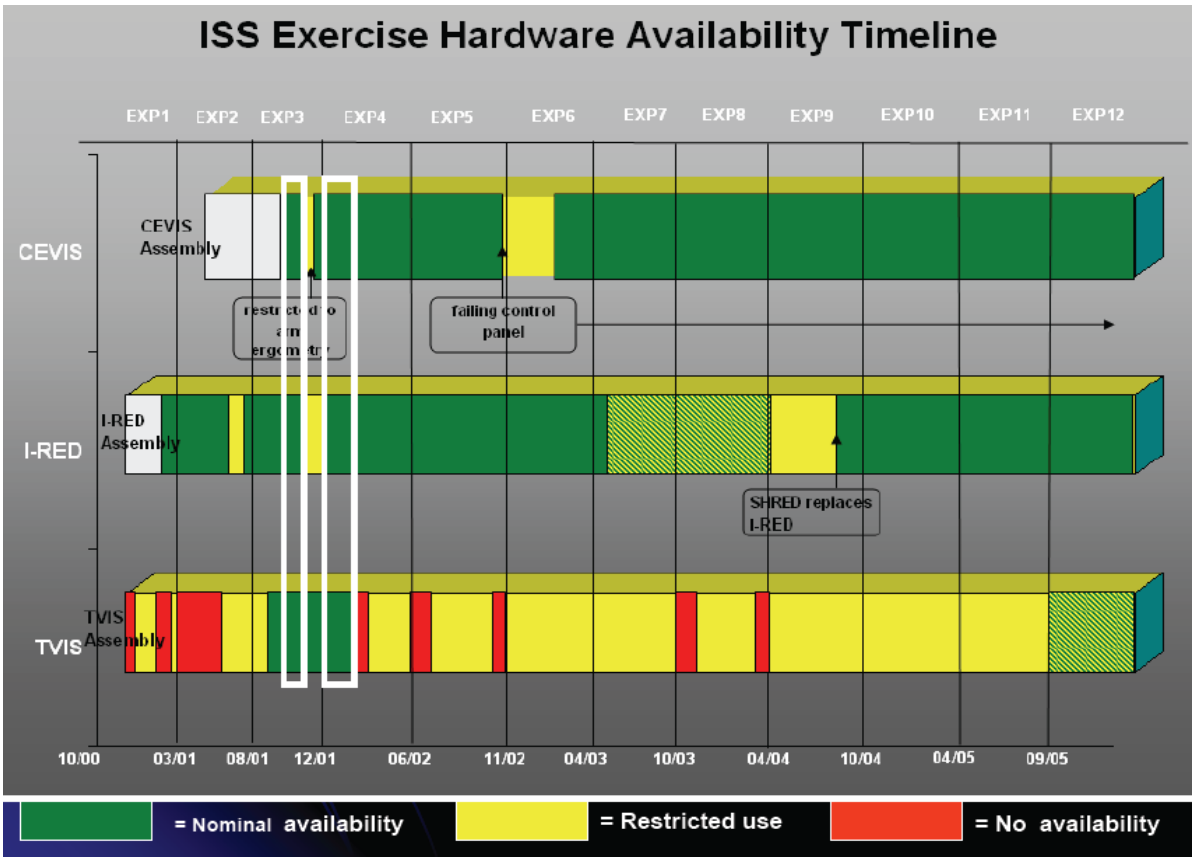

Figure 22. *Exercise equipment failures and other constraints have limited the access of ISS crewmembers to the full complement of aerobic and resistance exercise protocols. Full capability for all three devices was present only for two short windows during Expeditions 3 and 4 (tall white rectangles).*

group were unchanged. In short, metabolic adaptations in skeletal muscle appear to be less sensitive to unloading than do structural and functional changes related to morphology and contractility. Furthermore, countermeasure strategies that are insufficient to fully protect muscle from unloading-induced atrophy appear to be more effective in protecting against changes to the metabolic phenotype of the muscle.

These two major studies point to the need for load intensity if preservation of muscle mass and strength is to be accomplished. In these early years of the ISS, both hardware capabilities and reliability certainly contributed to this condition not being met. The iRED science requirement was to provide a load of up to an equivalent of 600 lb. (273 kg), but as mentioned above, the delivered hardware product provided only about half that amount. Ground-based studies have shown that it does produce a positive training effect similar to equivalent free weights when used in a high-intensity program [130], but it was thought to likely not provide sufficient load in a zero-gravity environment to prevent loss of muscle and bone tissue, as determined from parabolic flight studies [129]. For whole-body resistance exercises such as the squat, one's own body weight contributes a significant amount of load in a 1-g environment. In the weightlessness of space this contribution is lost. For this reason, load capacities for resistance exercise devices in space must be able to replace the body loads that are lost in the microgravity environment on top of the normal loads that one would use on the ground. Other problems in meeting load requirements were related to failures of the onboard exercise hardware with reduced utilization at other times, as well as use restrictions imposed due to transmission of forces into the structure of the space station itself. In fact, during the first 11 ISS Expeditions, there were only two short periods during Expeditions 3 and 4 when all three U.S. onboard exercise devices (CEVIS, TVIS, and iRED) were capable of being used under nominal conditions (Figure 22). The almost continuously suboptimal availability of exercise equipment likely has had a negative impact on maintenance of crew physical fitness during this time.

Nutritional regulation of protein metabolism as it pertains to maintenance of muscle mass is a growing research topic with implications for aging populations and those undergoing unloading, such as the ISS crew. Numerous investigations have addressed the roles of protein and amino acid intake in bed rest analogs for long-duration space flight (see below); whereas space flight data are much more limited. Aboard the ISS, protein intake has well-exceeded the U.S. Recommended Dietary Allowance (0.8 g/kg/d) both in the past (1.1 g/kg/d) and more recently (1.4 g/kg/d)[131]. Total caloric intake has historically been a problem; Stein et al. [132] reported significant decreases in body mass and protein synthesis after long-duration space flight on Mir. The reduction in protein synthesis was positively correlated with a decrease in energy intake during flight (r^2=0.86) [132]. These findings demonstrate the synergistic, deleterious effect of reduced energy intake on skeletal muscle metabolism and mass during mechanical unloading. A more detailed discussion of these topics can be found in the Nutrition Evidence Report [133].

As of spring 2022, crew evaluations for pre-postflight measures of body composition, isokinetic strength, and functional fitness are a part of crew medical requirements. Body composition is measured via dual x-ray absorptiometry (device manufacturer), preflight and postflight. Isokinetic strength testing is conducted at L-9/6 months, L-3/1 months, R+5, R+15, and R+30. Isokinetic testing is conducted using the Biodex. Primary movements include concentric knee extension and flexion (60 degrees/sec, 180 degrees/sec), concentric ankle plantar and dorsi flexion (30 degrees/sec), eccentric ankle plantar and dorsi flexion (30 degrees/sec), and trunk extension and flexion (90 degrees/sec) (note: only knee and ankle testing are conducted at R+5 due to lower back sensitivities following weightlessness). Functional fitness testing is conducted to evaluate different measures of whole and upper body strength and neuromuscular readaptation to space flight. Testing methodology is detailed in section J.

v) *Muscle Strength and Endurance Means Pre- to Postflight*

Muscle concentric isokinetic leg strength declined by 28% after long (110–237) and short (7) day flights. When exercise countermeasures were used during Skylab 2, it was reported that after

a 28 day flight, leg extensor strength was reduced by 25%; however, a less severe decline in strength was found for the upper extremities [134]. The most updated knowledge on long-duration

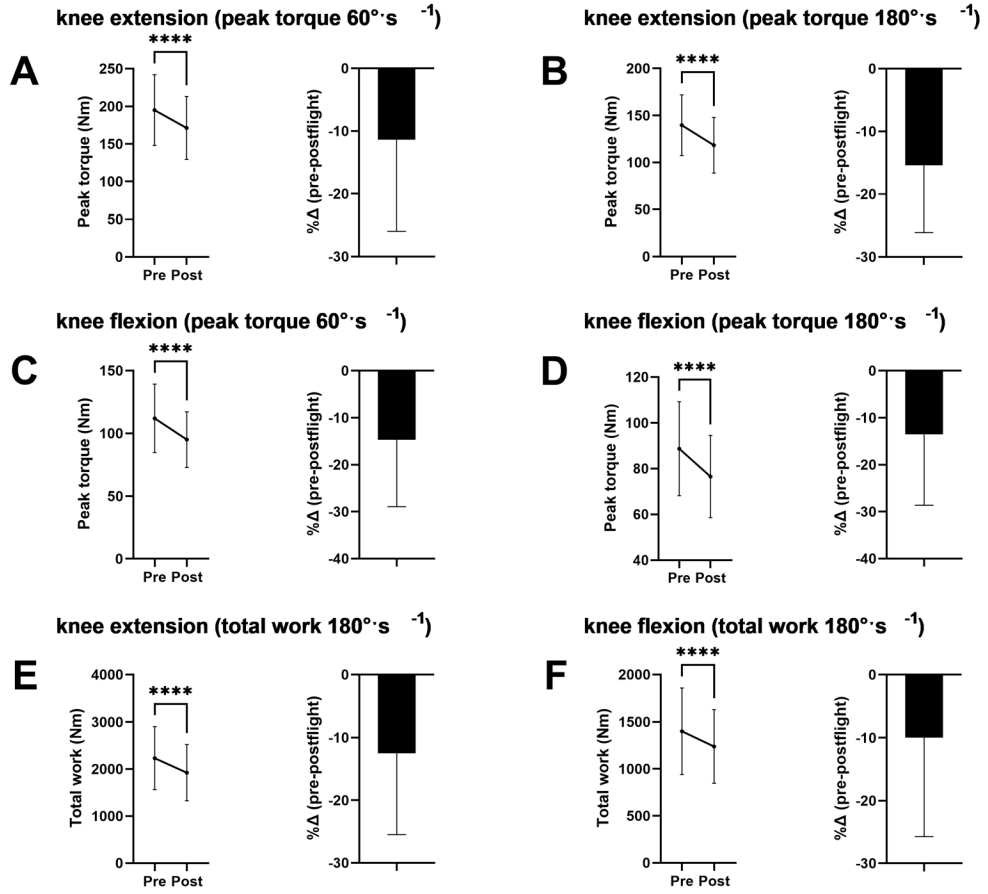

Figure 24. *Indices of leg muscle strength and endurance. Peak strength for knee extension (A, B) and knee flexion (C, D) and muscle endurance for knee extension (E) and knee flexion (F) are all reduced from pre- to postflight. ISS dataset pre- to postflight (L-3/1mo to R+5d), mission duration 174±37 days, n=98 (male 76/ female 22), Mean ± SD, **** = P<0.0001*

ISS missions reported that mean isokinetic strength declined 6–17% following spaceflight. One month after return to Earth, strength had improved, but small deficits of 1–9% persisted [52, 59]. NASA JSC preliminary unpublished data reports on the absolute and relative changes pre to post spaceflight in a larger sample, (n=98) finding that knee strength is reduced by about 14%, knee endurance is reduced by about 11% (Figure 24), and ankle strength is reduced by about 13% (Figure 25).

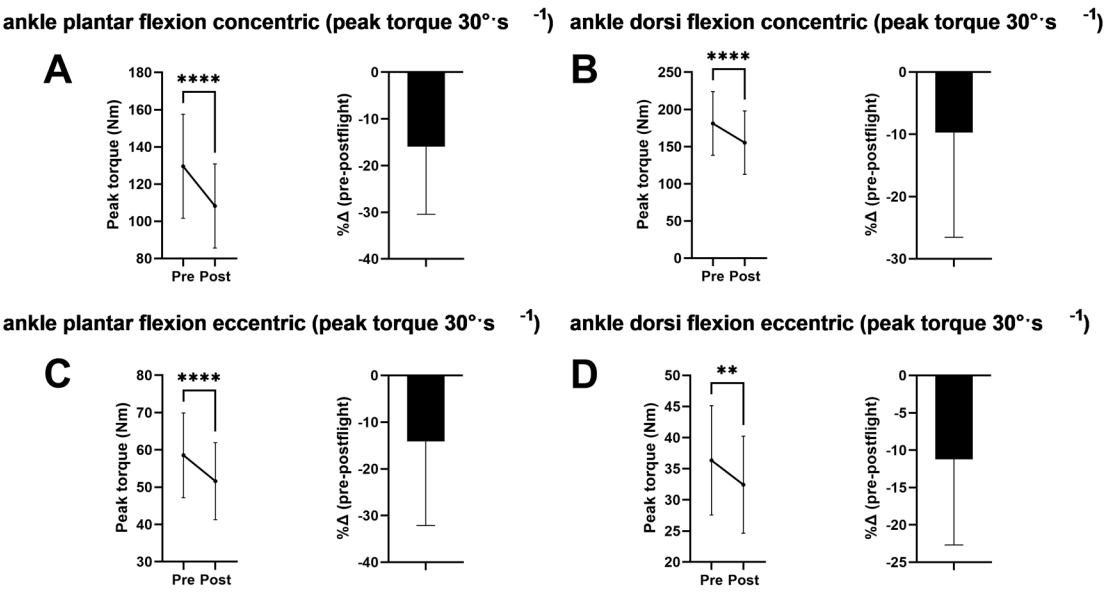

Figure 25*. Indices of ankle muscle strength. Peak strength for flexion concentric contraction of the ankle plantar (A) and ankle dorsi (B) and for flexion eccentric contraction of the ankle plantar (C) and ankle dorsi (D) are all reduced from pre- to postflight. ISS dataset pre- to postflight (L-3/1mo to R+5d), mission duration 174±37 days, n=98 (male 76/ females 22), Mean ± SD, **** = P<0.0001*

vi) Muscle Strength and Endurance Pre- to Postflight Individual Variability

The characterization of a responder from exercise training is described as an individual that does respond positively to exercise training. This means that they improve fitness (aerobic capacity or strength) levels after an exercise intervention. A non-responder is characterized as an individual that shows no improvements after the same exercise intervention as someone that does respond positively. In the context of spaceflight, the primary objective to exercise countermeasures is to maintain fitness over the course of spaceflight and upon return to Earth. Therefore, for this evidence report, the definition of a responder is a crewmember who maintains fitness from pre- to postflight, while a non-responder is one who does not maintain fitness from pre- to postflight outside of the measurement error. The major assumption in this characterization is that both responders and non-responders are completing the same nominal ISS exercise prescription.

Figures 26 and 27 provide preliminary ISS data on the characterization of the responders and non-responders to in-flight exercise prescription (same sample as Figures 24 and 25). Instrument variation has been reported to be velocity (±1 deg/s), angle (±0.4%, ±1 deg), and torque (±0.9%) [135], and the test-retest reliability of isokinetic knee extension and flexion is reported to be reliable (ICC range, >0.90) [136].

ISS data also suggest (figures 26 and 27) only 15–18% of crew population respond well (i.e., improve fitness) to the exercise prescription in-flight that maintain knee muscle strength (15%), knee endurance (18%), and ankle muscle strength (17%) within the measurement error. In some cases, crew do return with improvements in muscle strength and endurance. Notably, 11–14% of the current crew population improve knee strength (11%) and endurance (14%) and ankle strength (13%). However, over half (55–63% of the sample) crew lose greater than 10% of their

preflight knee strength (63%) and endurance (55%) and ankle strength (57%). Of the entire crew population within this dataset, 22-31% of crew lose greater than 20% of their preflight knee strength (31%) endurance (22%) and ankle strength (26%) upon return to Earth.

Understanding and improving the dose of resistance exercise will be needed to make sure all crew return safely, while maintaining muscular health and performance. Moreover, future long-duration missions (i.e., Artemis) to the Lunar and Martian surface will require adequate countermeasures to maintain fitness to complete critical mission task (e.g., return to Earth and planetary egress, and space walking with suits).

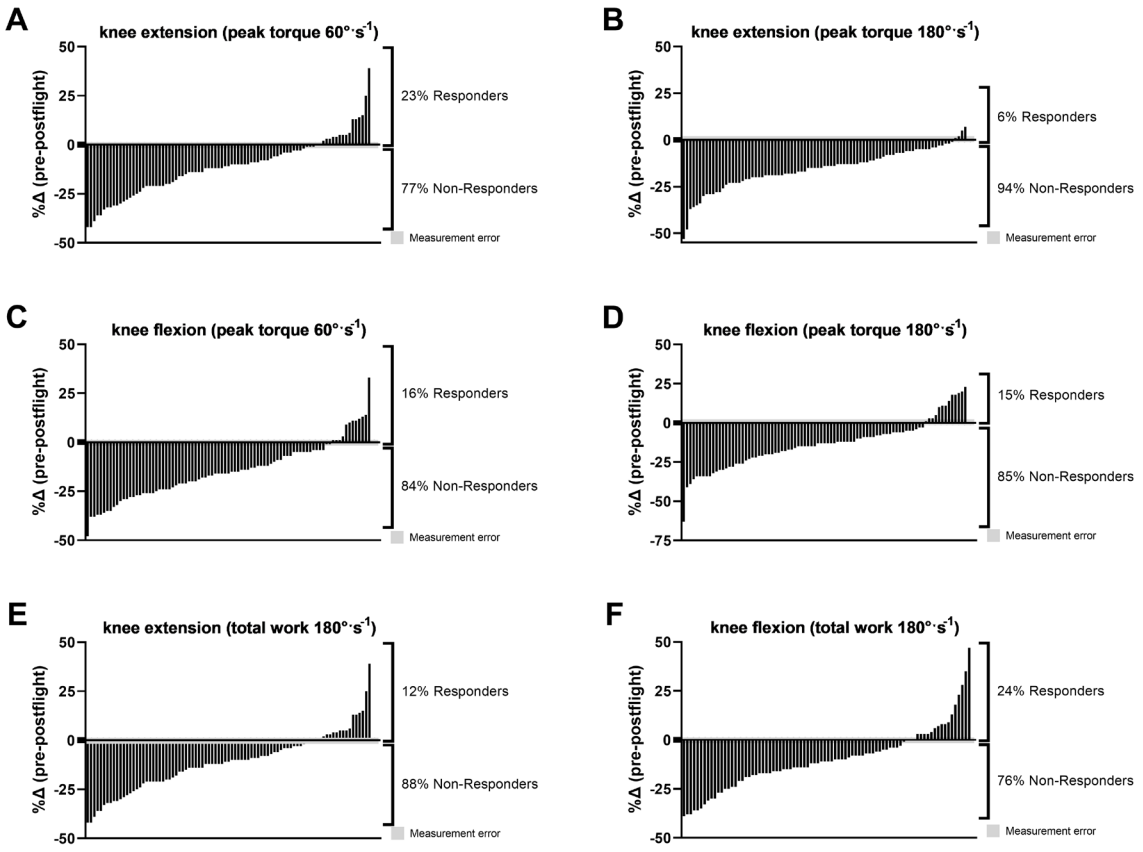

Figure 26. *Individual variability on indices of muscle strength and endurance. Between 75–94% of crew do not respond to the current exercise interventions for maintaining muscle strength and endurance for peak strength for knee extension (A, B) and knee flexion (C, D) nor for muscle endurance for knee extension (E) and knee flexion (F). Responder is defined as maintaining preflight fitness within instrumentation error (gray box ±1%). ISS dataset pre- to postflight (L-3/1mo to R+5d), mission duration 174±37 days, n=98 (male 76/ females 22)*

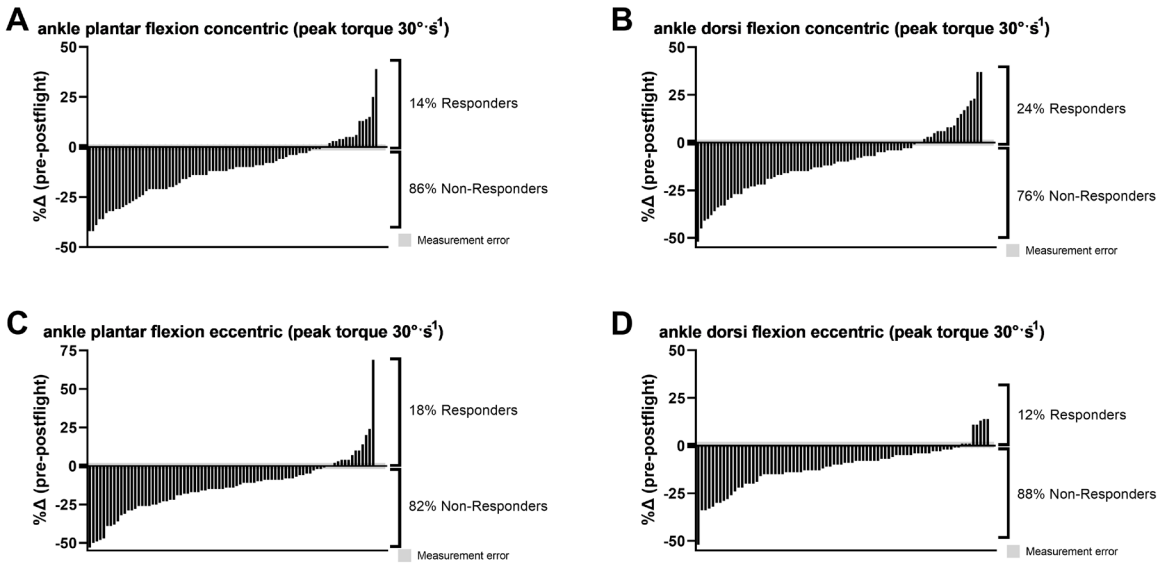

Figure 27*. Individual variability on indices of ankle muscle strength. Between 75–88% of crew do not respond to the current exercise interventions for maintaining muscle strength for flexion concentric contraction of the ankle plantar (A) and ankle dorsi (B) nor for flexion eccentric contraction of the ankle plantar (C) and ankle dorsi (D). Responder is defined as maintaining preflight fitness within instrumentation error (gray box ±1%). ISS dataset pre- to postflight (L-3/1mo to R+5d), mission duration 174±37 days, n=98 (male 76/ females 22)*

vii) *Muscle Functional Fitness Means Pre- to Postflight*

Decreases in strength are problematic due to the functional limitations they impose. In a novel study utilizing a weighted suit to reduce subjects' relative strength, Ryder et al. [137] determined strength and power thresholds below which functional task performance were impaired. However, there is high variability in performance on tasks incorporated in the functional fitness test. Laughlin et al. [138] reported that percent change was reduced from before (L-60 day) to postflight (R+5-7 days) performance for hand grip strength (−4.9±8.6%), flexibility (sit and reach: −8.0±9.5%), agility and coordination (time increased to complete cone test: +11±11.8%), upper body endurance (pushup: +3.1±47.0%, pull up: −0.6±41.2%), abdominal strength (sliding crunches: −0.09±16.1%), upper body strength (bench press: 0.68±9.9%), and lower body strength (leg press: −2.8±7.2%) [138]. The sample size for this test was between 24–34 crew. The ISS data in Figures 28 and 29 increased the sample size to 67 crew compared to the Laughlin et al. study at the preflight time point and find similar results [138]. Specifically, hand grip strength (right: −3±6%, left: −4±6%), flexibility (sit and reach: −7±10%), agility and coordination (cone test: +10±10%), upper body endurance (pushup: −3±22%, pull up: −2±35%), abdominal strength (sliding crunches: 4±20%), upper body strength (bench press: 3±8%), and lower body strength (leg press: −4±7%) are improved or maintained in some crew.

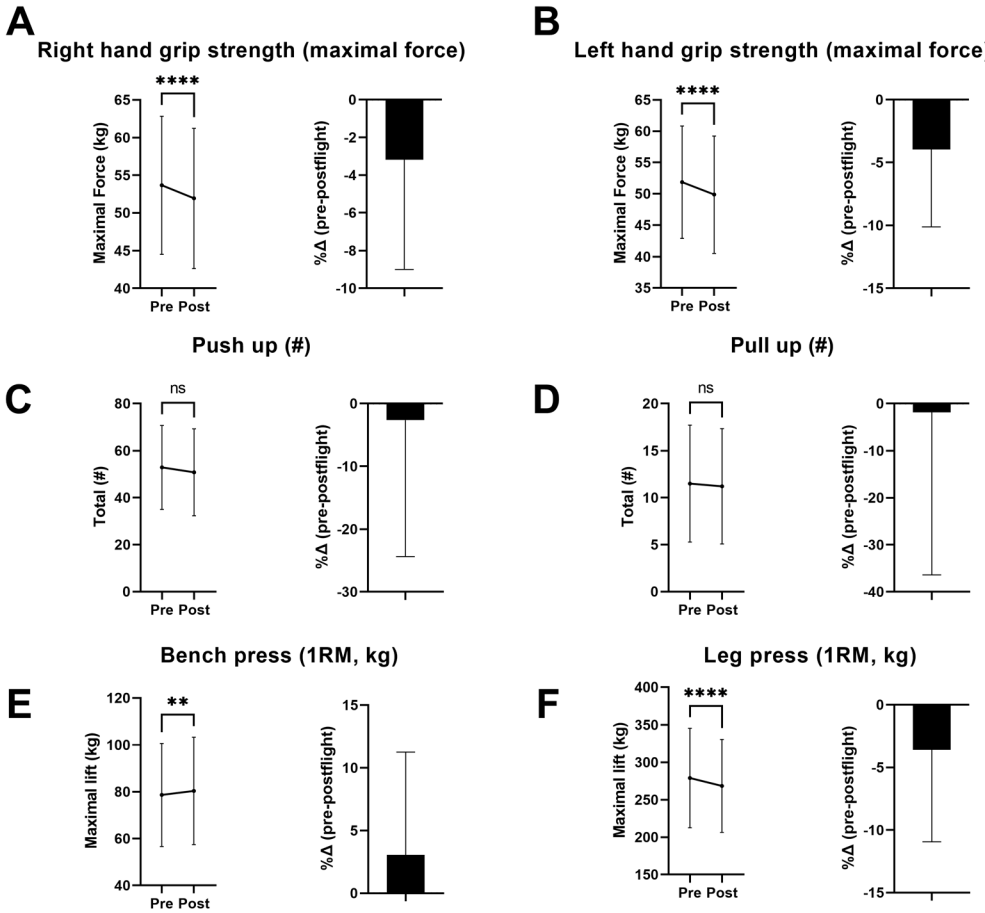

Figure 28. *Indices of functional fitness. Left (A) and right (B) handgrip strength and upper (E) and lower (F) body strength are reduced from pre to postflight, whereas upper (C, D) body endurance is similar. ISS dataset pre- to postflight (L-2mo to R+7d), mission duration 175±39 days, n=67 (male 36/ females 18), Mean ± SD, **** = P<0.0001*

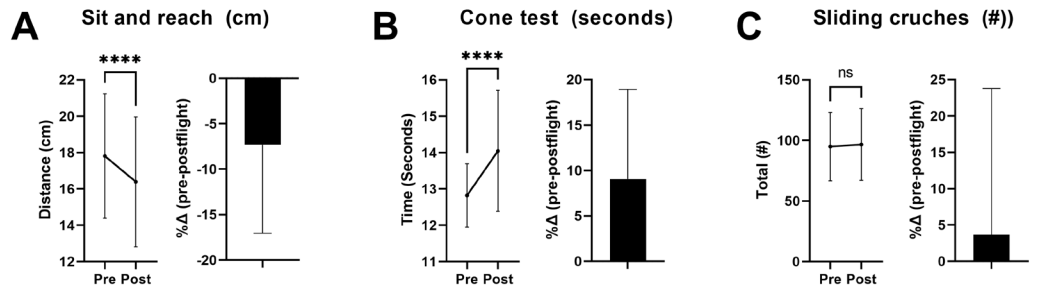

Figure 29. *Indices of functional fitness. Sit and reach flexibility (A) and cone test agility (B) functional fitness are reduced from pre to postflight, whereas sliding crunches (C) abdominal endurance is similar. ISS dataset pre- to postflight (L-2mo to R+7d), mission duration 175±39 days, n=67 (male 36/ females 18), Mean ± SD, **** = P<0.0001*

viii) *Muscle Functional Fitness Pre- to Postflight Individual Variability*

As mentioned in the previous section, the characterization of a responder from exercise training is described as an individual who does respond positively to exercise training. This means that they improve fitness (aerobic capacity or strength) levels after an exercise intervention. A non-responder is characterized as an individual who shows no improvements after the same exercise intervention. In the context of spaceflight, the primary objective to exercise countermeasures is to maintain fitness over the course of spaceflight and upon return to Earth. Therefore, for this evidence report, the definition of a responder is a crewmember who maintains fitness from pre- to postflight. The definition of non-responder is a crewmember who does not maintain fitness from pre- to postflight outside of the measurement error. The major assumption in this characterization is that both responders and non-responders are completing the same nominal ISS exercise prescription.

One repetition maximum strength is reported to have good to excellent test-retest reliability with an intraclass correlation coefficient generally high for young to middle aged adults (ICC >0.98) [139]. A systemic meta-analysis reported that 66% of studies find no difference between pre and post repeat testing; however, it has also been reported that 34% of studies of this meta-analysis have higher strength values on the retest by about 5.5 kg for lower body exercise and 1.8 kg for upper body exercise [139]. Among our ISS crew dataset, this was ±2% of one repetition maximal for upper and lower body strength test. We conservatively used ±2% as within the measurement error for defining responders and non-responders for all the functional fitness tests.

ISS preliminary data (Figures 30 and 31) show high variability among functional fitness measurements for the crew population that responds (11–58% of sample) for a particular measurement to the in-flight exercise prescription that maintains fitness (i.e., responders). Specifically, hand grip strength (right: 40%, left: 44%), flexibility (sit and reach: 31%), agility and coordination (cone test: 19%), upper body endurance (pushup: 61%, pull up: 56%), abdominal strength (sliding crunches: 63%), upper body strength (bench press: 78%), and lower body strength (leg press: 48%) were maintained or improved in some crew.

Particular measurements do improve for some crew (9–50% of sample) from pre- to postflight. Specifically, hand grip strength (right: 16%, left: 9%), flexibility (sit and reach: 11%), agility and coordination (cone test:13%), upper body endurance (pushup: 50%, pull up: 40%), abdominal strength (sliding crunches: 49%), upper body strength (bench press: 58%), and lower body strength (leg press: 16%) are improved from pre-flight measures.

On the other hand, some crew (8–37% of sample) have loss in functional fitness of greater than 10% pre- to postflight. Specifically, hand grip strength (left 12%, right: 19%), flexibility (sit and reach: 34%), agility and coordination (cone test: 32%), upper body endurance (pushup: 31%, pull up: 37%), abdominal strength (sliding crunches: 26%), upper body strength (bench press: 8%), and lower body strength (leg press: 21%) are reduced by ≥10%.

Of critical importance, some crew (1–24% of sample) have loss in functional fitness of greater than 20% pre to postflight. Specifically, hand grip strength (left and right: 1%), flexibility (sit and reach: 10%), agility and coordination (cone test: 11%), upper body endurance (pushup: 20%, pull up: 24%), abdominal strength (sliding crunches: (5%), upper body strength (bench press: 1%), and lower body strength (leg press: 1%).

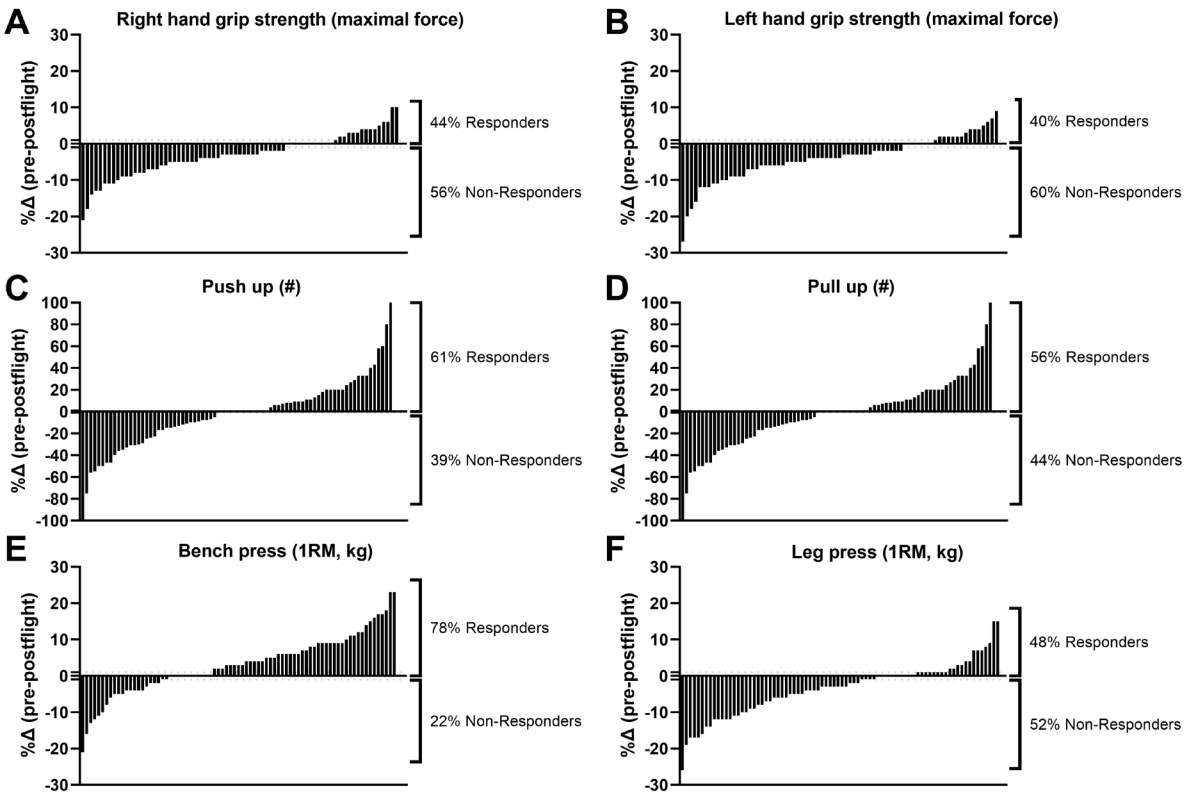

Figure 30. *Individual variability on indices of functional fitness. Between 25–70% of crew do not respond to current exercise interventions for maintaining right (A) and left (B) hand grip strength, upper body muscle endurance (push up (C) and pull up D)), and upper (E) and lower (F) body strength. A responder is defined as maintaining preflight fitness within test-retest repeatability of measurement (±2%). ISS dataset pre- to postflight (L-2mo to R+7d), mission duration 175±39 days, n=67 (male 36/females 18)*

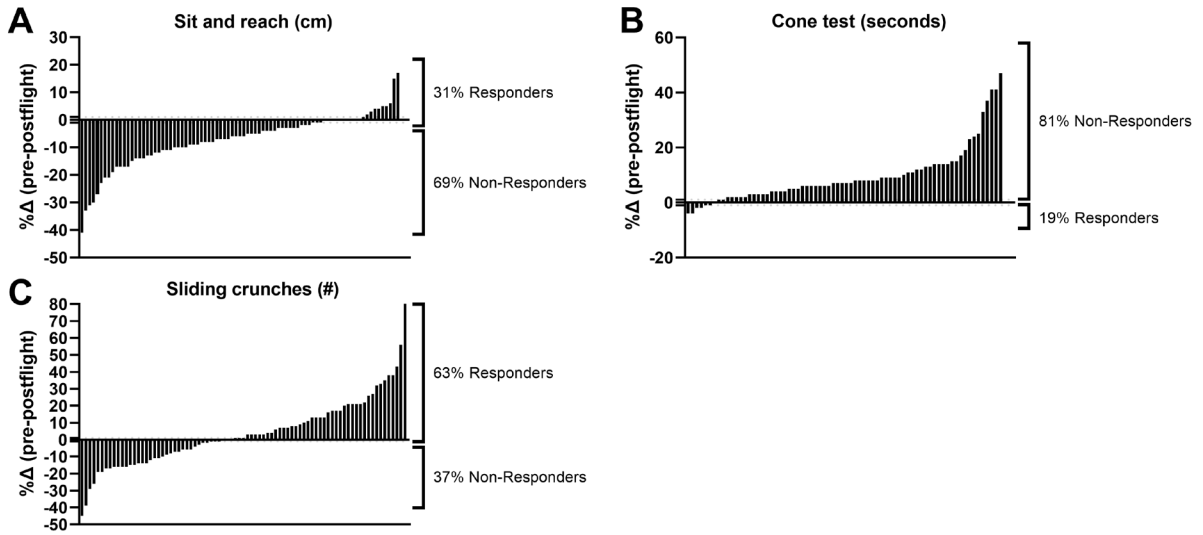

Figure 31. *Individual variability on indices of functional fitness. About 19-69% of crew do not respond to current exercise interventions for maintaining sit and reach flexibility (A), agility (B) and abdominal endurance (C). A responder is defined as maintaining preflight fitness within test-retest repeatability of measurement (±2%). ISS dataset pre- to postflight (L-2mo to R+7d), mission duration 175±39 days, n=67 (male 36/ females 18)*

ix) Exercise Countermeasures

ISS crewmembers perform treadmill, cycle ergometer, and resistive exercise during their missions to counter the effects of long-duration space flight exposure on muscle, bone, and cardiovascular fitness (Figure 32). An exercise session of 2.5 hrs per day is scheduled and includes time to change into exercise clothing and clean up, totaling the effective daily exercise time to approximately 1 hr 15 min. Typically, crewmembers perform aerobic and resistance exercise 6 days per week; however, the exercise prescriptions and adherence to the prescriptions have varied widely between crewmembers. The Astronaut Strength, Conditioning, and Rehabilitation (ASCR) group has worked in more recent missions towards standardizing exercise prescriptions. Further details regarding exercise prescriptions are in section q. Exercise countermeasures. It is important to note that exercise in 0g is less than that on 1g. For example, when evaluating foot forces the three exercise devices studied (CEVIS, TVIS, and iRED) were not able to elicit loads comparable to exercise on Earth, with the exception of CEVIS at its maximal setting [128]. Thus, greater exercise hardware loads are required to elicit comparable 1g stress on skeletal muscle and bone and cardiorespiratory systems. Each exercise device is mounted on a vibration isolation system to protect the structural integrity of the ISS by minimizing the transfer of force generated during exercise to the station. The following will briefly overview the hardware capabilities for aerobic (TVIS, T2, CEVIS) and resistive exercise (iRED and ARED).

Treadmill with vibration isolation and Stabilization (TVIS). The TVIS designed and constructed by NASA was used for aerobic exercise on ISS. The belt consists of 160 1.3 cm aluminum slats suspended across two parallel sets of 25 roller bearings and wrapped around the front and rear drums of the treadmill. The running surface is 33 cm (13 in) wide and 112 cm (44 in) long. The treadmill can be operated in the motorized or non-motorized modes. In the motorized mode, the belt speed is adjustable from 0.0 to 16 $km \cdot h^{-1}$ (0.1 to 10.0 mph) in 0.16 $km \cdot h^{-1}$ (0.1 mph) increments. In the non-motorized mode, the belt speed is dependent upon the effort provided by the exerciser, and the resistance to belt motion can be manipulated with a braking

system. Upon evaluation on ISS, the maximum on-orbit single-leg loads from TVIS were 1.77 body weight (BW) while running at 8mph, and on average, foot forces were 77% for walking, 75% for running of the foot forces compared to 1g [128]. On orbit, the crewmember wears a shoulder and waist harness and is pulled to the treadmill surface using either metal cables attached to pre-loaded springs or bungee cords. On the ISS, the TVIS is suspended within an opening in the floor and allows limited movement in six degrees of motion. Movement of the TVIS is counteracted with active (gyroscope and stabilizers) and passive (wire ropes) vibration isolation systems. These systems are intended to minimize the dynamic forces of exercise being transferred to the structure of the ISS Service Module while maintaining a relatively stable exercise surface.

The second-generation treadmill (T2) was delivered to ISS in 2010, respectively. TVIS was replaced in 2010 by the Combined Operational Load Bearing External Resistance Treadmill (COLBERT), technically named the Treadmill 2 (T2). This second-generation treadmill was derived from a commercial Woodway Path treadmill and has a redesigned passive vibration isolation system. Improved functional capabilities include a greater maximum speed (up to 5.5 m/s). Use at low-speed results in significant instability and tends to saturate the vibration isolation system and impart higher-than-desired loads to the vehicle. The T2 allows astronauts to run up to 12.4 mph with higher percentages of body weight loading due to improvements in the harness comfort. Astronauts have used both the TVIS and T2 in the non-motorized and motorized modes.

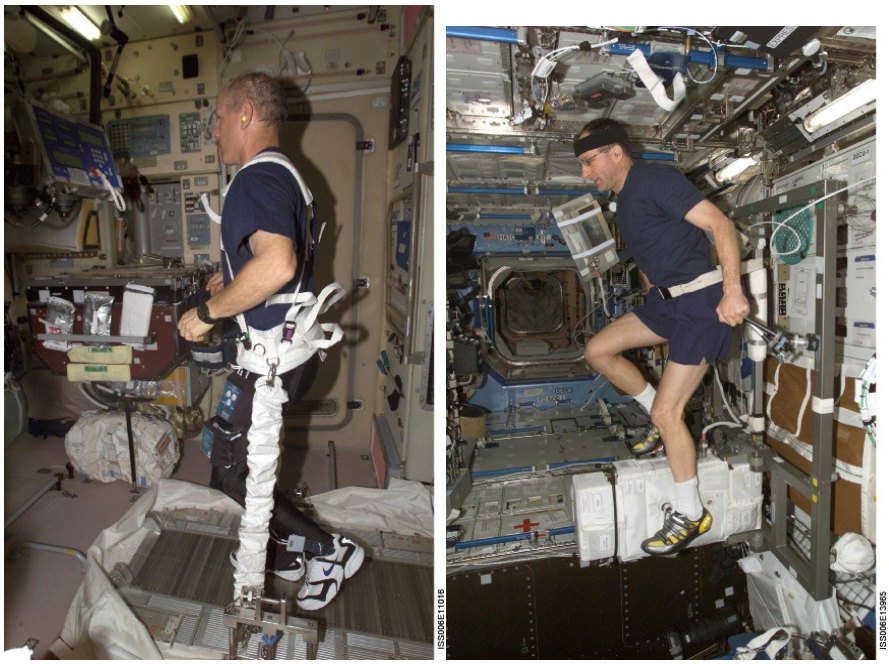

Figure 32. *International Space Station crewmembers exercising on the treadmill with vibration isolation system (TVIS, left) and the cycle ergometer (CEVIS, right).*

Cycle Ergometer with Vibration Isolation and Stabilization (CEVIS). CEVIS was installed on the ISS in early 2001 to provide aerobic and cardiovascular conditioning; it is also used to conduct periodic fitness evaluations of aerobic capacity. The cycle ergometer with vibration isolation system operates from 0 to 350 W and allows pedal speeds from 0 to 125 revolutions per minute. Exercise prescriptions are uploaded to the control panel, and data are downloaded by ground personnel through the station's network server. The control panel records resistance, speed, and

torque, from which power output can be calculated. An integrated heart rate monitor synchronizes performance data with heart rate, similar to a stationary mechanical bicycle, and is connected to the ISS with wire tethers. It sits on a vibration isolation system which reduces impacts to the structure of the ISS. Astronauts snap their shoes onto the pedals and use a seatbelt to hold them on the bicycle. They can change the workload to maximize their workout. The speed is also adjusted to keep the astronaut's heart rate at a specific target, tracked with a heart rate monitor. Forces during CEVIS exercise were small, approaching only 0.19 BW at 210W and 95RPM.

Interim Resistive Exercise Device (iRED). IRED (Figure 33) contained a series of 16 flex packs stacked vertically designed to provide resistance training. The flex packs revolve about a metal axle. When the metal axle is turned, the rubber spokes are stretched, increasing the resistance offered by the device. A nylon cord was attached to a spiral pulley at the bottom of the flex pack column that allows a gearing mechanism to rotate in the same direction as the rotating flex packs. The gear is engaged or disengaged by a lever on the outside of the casing of the iRED. When engaged, the gear is turned by a hand-crank located on the top of the iRED, which rotates the splines between the flex packs counterclockwise, stretching the rubber flex packs. When the rubber is stretched, or preloaded, the resistance provided is dependent upon the degree of rotation of the inner hub of the flex packs. The resistance level is displayed via a set of 12 indicator marks on the side of the can. Each iRED canister is loaded independently. Once connected to a spiral pulley, the discs provide up to 300 lbs. of linear resistance. While on-orbit, crewmembers could complete daily exercise protocols, including squats, to load the spine, hips, and legs, which are most affected by microgravity. iRED evaluation of ground reaction force during a parabolic flight reported peak force and total work were significantly less, by 42–46% and 33–37%, compared 1g to 0g for the iRED [92]. This data suggest that crew must exercise at greater external resistive forces in-flight compared to on Earth. The largest single-leg forces during resistance exercise were 0.72 BW during single-leg heel raises and 0.68 BW during double-leg squats [128].

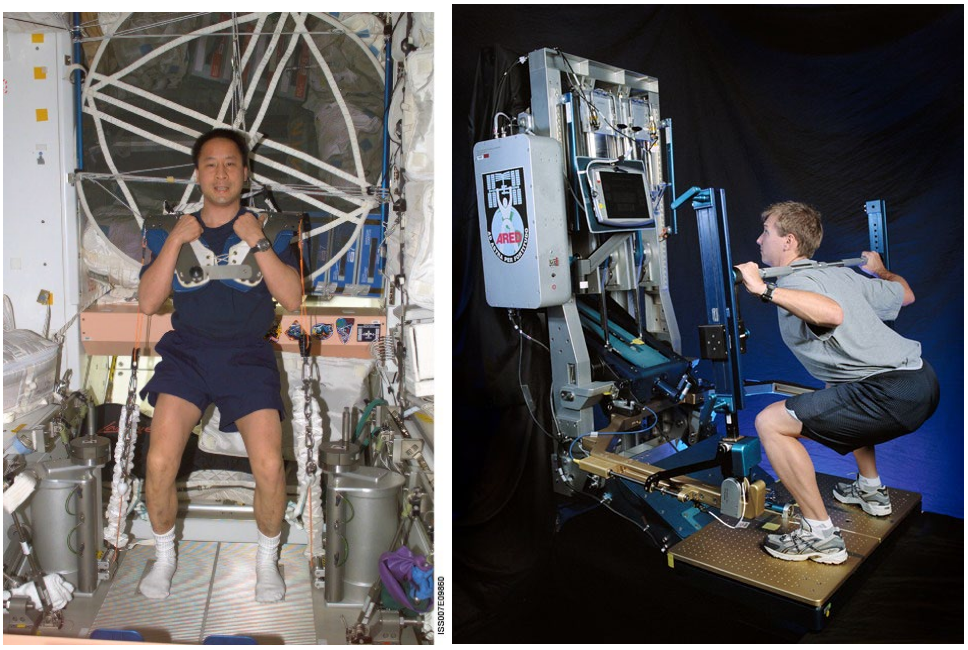

Figure 33. *International Space Station crewmembers exercising on interim resistive exercise device (iRED, left) and advance resistive exercise device (ARED).*

Advanced Resistance Exercise Device (ARED). The interim Resistive Exercise Device (iRED) (Figure 32) was replaced with the Advanced Resistance Exercise Device (ARED) in 2008. ARED uses adjustable resistance piston-driven vacuum cylinders along with a flywheel to system to provide loading for crewmembers to experience load and maintain muscle strength and mass during long periods in space. The ARED provided improvements from the iRED in both maximum loading capabilities and loading characteristics. The ARED can provide concentric loading up to 600 lbs. and 250 lbs. for cable exercise, an eccentric-concentric ratio of ~90%, and constant force throughout the range of motion using inertial flywheels. In comparison, the iRED was limited to 300 lbs., eccentric to concentric ration of only 60-80%, and load was not constant through the range of motion [140]. Advanced resistive exercise device accommodates individuals from the fifth to 95th percentile in size. An exercise cable offers focused upper-body and lower-body strength training at loads. Hardware attachments include an exercise bench with belt, heel-raise plat-form, cable pull bar, cable pull handles, and ankle cuffs. ARED consists of seven distinct assemblies:

- Exercise Platform Subassembly mounts to the ARED structural frame and provides the surface from which to perform exercises. The platform houses two force plates, with four load cells installed under each plate to measure the reactive loads for all exercises.
- Cylinder/Flywheel Assembly generates the loads for all exercises. The vacuum canisters provide the primary force while the flywheels provide the simulated inertial component of the exercise as would be experienced on the ground. These are mechanical assemblies only.
- Main Arm Assembly includes the wishbone arm and the lift bar components. Load cells were installed in the lift bar struts.
- Arm Base Assembly includes the load adjustment mechanism, interfaces for the Cylinder/Flywheel Assembly, Main Arm Assembly, Cable Pulley Assembly, and the Frame/Platform Assembly. These assemblies contain two load cells and one rotational sensor. The two load cells measure the reactive loads during cable-based exercises.
- Belt/Pulley Assembly provides the capability to perform cable-based exercises. It provides the interfaces between the exercise rope and the Arm Base Assembly via the Cable Arm Ropes to provide load for the exercises. This is a mechanical assembly only.
- Exercise Bench Assembly is an accessory that mounts to the platform and provides a surface for performing shoulder presses, bench presses, and other seated or lying exercises. Sit ups and other core exercises can be performed using the bench as well. It is folded up and stowed when not in use.
- Heel Block Assembly is an accessory that mounts to the platform and allows the capability for performing heel-raise exercises. It is removed and stowed when not in use.

Custom ARED software receives prescriptions from the ground, displays the workout to the crewmember, and records data. The data acquisition system on ARED was designed to provide repetition counts, sets completed, static and dynamic load measurements, and exercise start and stop times. Because of malfunctions in the instrumentation system, these data are often self-reported. A new instrumentation box is due to be implemented in 2022 to update the previous 2016 version. Force-plate measure exercise loads, more accurately track resistance exercise performance, and accommodate biomechanical analyses [141].

All United States Orbital Segment (USOS; NASA, Japan Aerospace Exploration Agency European Space Agency, and Canadian Space Agency) crewmembers undergo specific medical requirements testing before and after their ISS missions. Part of this testing includes isokinetic muscle strength and endurance testing of the legs and ankle muscles evaluated preflight and at 5-7 days after landing. In Figure 35, we present ISS isokinetic strength and endurance testing results as percent change from preflight baseline. Results are divided into two groups that exercised on iRED and on ARED hardware and find that no differences were observed. This preliminary data suggest that hardware differences did not influence pre- to post flight changes in muscle strength. This is in agreement with English et al. [59] that reports similar changes regardless of resistance hardware (Figure 34). This data also supports that exercise prescriptions have important implications for muscle strength. However, it is important to note that maximal capabilities of hardware may limit those crew with higher than average strength if exercise prescription is limited. For example, if crew with higher-than-average strength meet or exceed the maximal capabilities of the hardware, appropriate stress and adaptations will not occur.

The current permissible outcome limit for muscle strength in returning crewmembers is at or above 80% of baseline values (NASA Space Flight Human System Standard Volume 1: Crew Health; NASA-STD-3001) [30] (Table 4). Both ARED-era and iRED-era crew have losses less than the 20% standard. Furthermore, an examination of the individual data shows that many individuals have lost more than the targeted 20% threshold. It is also important to keep in mind that the medical requirements testing is conducted approximately 1 week after landing and therefore may not reflect a crewmember's performance ability in the immediate post-landing time frame. Crew may have recovered some at the 1-week postlanding time-period. Moreover, the standard isometric tests differ in their capability to the functional fitness measurements. Forward Human Research Program work aims to develop more performance-based strength standards that can better be used as benchmarks for mission success. Doing so will not only aid in designing better exercise countermeasure strategies but will ultimately lead to greater assurance of crewmember safety.

Table 4. *Preflight Muscle Strength Requirements*

	Minimum	Microgravity EVAs	Celestial Surface EVAs	Unaided Egress
Deadlift	1.0 × body weight	1.3 × body weight	1.6 × body weight	1.3 × body weight
Bench press	0.7 × body weight	0.8 × body weight	1.0 × body weight	0.7 × body weight

From 4.6.1 NASA Space Flight Human System Standard Volume 1: Crew Health; NASA-STD-3001 [30]

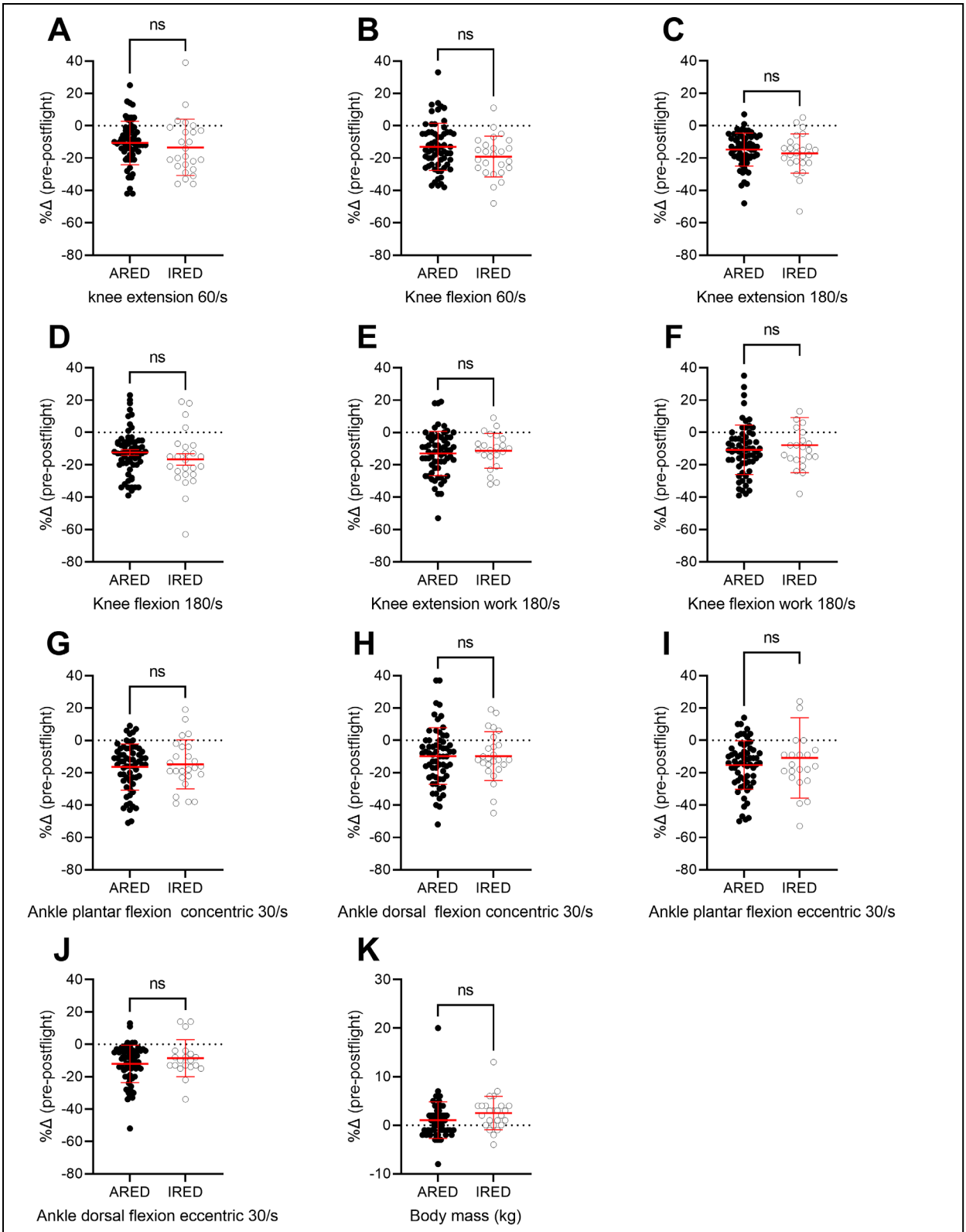

Figure 34. *Pre to post-flight changes (%) in isokinetic strength for knee extension (A-C,) and endurance (D) and ankle strength (G-J) and body mass (K) for long-duration ISS crewmembers using iRED or ARED during their flight. Total sample n= 90 at L-30mo, ARED sample, n=66 (male 82%, female 18%), iRED, n=25 (male 80%, female 20%). ns, not statistically significant at P<0.05. Mean ±SD*

A recent investigation examined the effects on body composition with use of iRED compared to the use of ARED onboard the ISS [131]. Eight iRED users and five ARED users were subjected to DXA analysis prior to flight and again anywhere from 5 to 45 days postflight (mean 12±11 days postflight). Total body mass was unchanged in both groups; however, lean body mass was increased in ARED users and fat mass was reduced. These data are consistent with the view that ARED use is better for musculoskeletal outcomes following ISS missions; however, the effect of space flight on postural skeletal muscles is difficult to assess via whole body lean mass, as the target tissues do not likely represent a large enough portion of the total lean mass pool to detect changes with sufficient accuracy. It appears that MRI and potentially ultrasound imaging technologies are required to adequately detect morphological changes associated with loss of muscles strength.

Monitoring exercise data. Exercise equipment and equipment availability have varied throughout the history of the ISS. For example, crewmembers have not always been able to exercise due to hardware failure or were only able to exercise at lower intensities to conserve station power resources. The ISS is outfitted with a suite of exercise hardware providing the ability to exercise at higher intensities with improved instrumentation for data monitoring and downlinking capabilities. Historically, exercise data was not well tracked due to poor heart rate data quality or sporadic use of the heart rate monitor by the crewmembers as well as difficulty syncing the data from the exercise devices to the heart rate monitors. However, exercise data reporting has been greatly improved over the last several years due to improved quality of heart rate data (although significant dropout still occurs) and because the new suite of exercise hardware is instrumented to record exercise loads and speeds. These improvements allow researchers and the NASA medical community to more accurately monitor the astronauts' adherence to the exercise prescriptions and track progressions in performance during the mission. These efforts will provide a greater understanding of the in-flight exercise intensity and volume requirements towards maintaining aerobic fitness.

MEDB 5.2 On-Orbit Strength & Conditioning Monitoring. This medical requirement assures that all crewmembers participate in an exercise program prescribed and scheduled during flight. The exercise prescription is recorded and adherence to the prescription is evaluated. This information is used to assess crew readiness for in-flight duties and EVA, assess countermeasure effectiveness, and provide a historical record of the exercise program performed during flight. Exercise prescriptions for each crewmember are updated as the mission progresses. Reports are provided to the surgeon regarding the exercise activities conducted during flight for each crewmember will be delivered every other week. A final summary report detailing all exercise conducted during flight and outcome measures from ground testing is also provided.

For all exercise modalities the following are recorded:

- Number of training sessions/week
- For Treadmill with Vibration Isolation and Stabilization (TVIS) and Treadmill 2 (T2): Speed of walking/running, subject load device settings, heart rate, exercise duration
- For T2: Ground reaction forces and impact loading
- For Cycle Ergometer with Vibration Isolation System (CEVIS): Work rate (W), pedaling speed, heart rate, exercise duration, arm or leg session
- For Resistive Exercise Device (ARED): Exercises performed, number of sets and repetitions, load settings

j. ISS Mission Testing Methodology

i) Aerobic Capacity Exercise Testing (VO_2peak and Ventilatory Threshold)

The aerobic capacity exercise test is currently a medical requirement titled MEDB 4.3 Clinical Stress Test and MEDB 4.1 Aerobic Capacity Test. These assessments are specifically the Cycle Ergometer Test/Aerobic Functional Capacity and are performed on an annual basis for all astronauts on active flight status, as well as for assigned crewmembers before, during and following ISS flight. As part of the Medical Requirement Integration Document (MRID), the annual assessments of aerobic capacity and tolerance are used for prognostic clinical cardiac abnormalities and for developing exercise training prescriptions, hardware efficacy evaluation, and rehabilitative program effectiveness, and are for research purposes. See Figure 35 for the testing schedule.

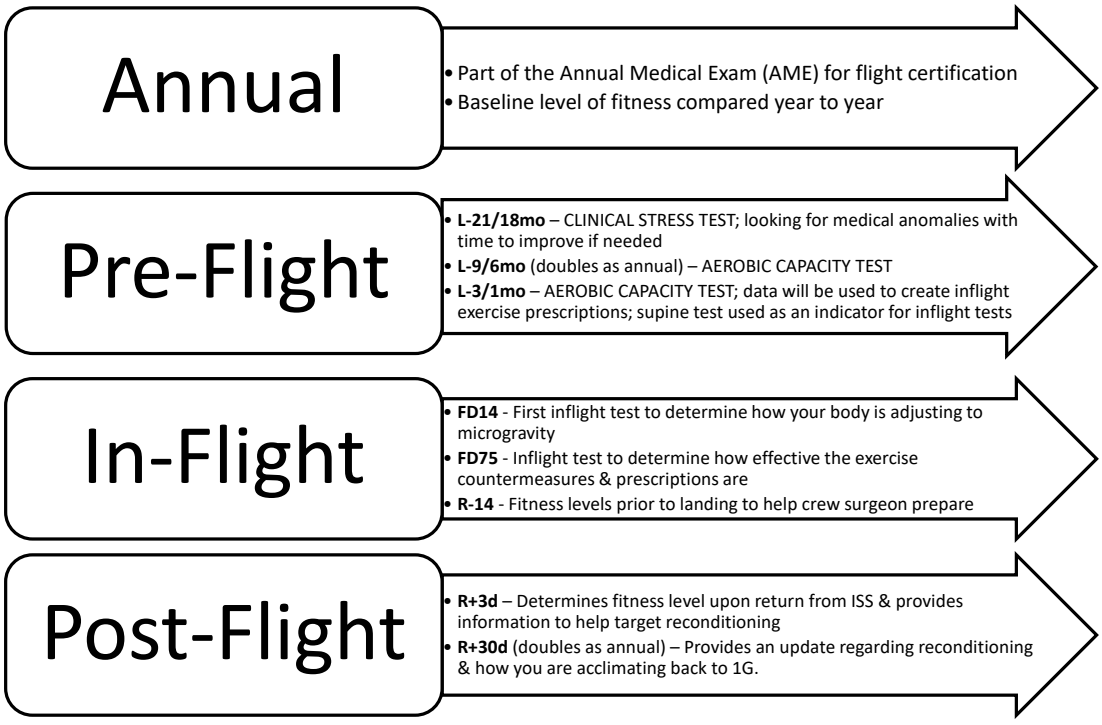

Figure 35. *Current schedule of pre, during, and postflight measurements of aerobic capacity exercise testing.*

The aerobic capacity exercise test, also termed VO_2max (and VO_2peak), is a validated index measuring the limits of the cardiorespiratory system and a "stress test" that measures the health of lungs, heart, and muscles, as well as the ability to transport oxygen from the atmosphere air to the muscle tissues for utilization during maximal exercise. The highest recorded value (volume of oxygen) during the end of the test at maximal aerobic exercise is expressed in absolute terms (L/min) relative to body mass ($mL \cdot kg \cdot min^{-1}$) and is defined as VO2peak. A true VO_2max is defined as plateau in VO_2 with an increasing workload; however, four of the five following criteria must be met to be considered maximal effort: (1) a respiratory exchange ratio (RER) of ≥1.10, (2) a plateau in VO_2 with increasing workloads, (3) workload volitional fatigue (a fall of 10 RPM), (4) exercise peak HR within 10 beats of the age-predicted maximal HR [207–(0.67 × age)], and/or (5) a rating of perceived exertion (RPE) at or greater than 19 on a 20 point scale.

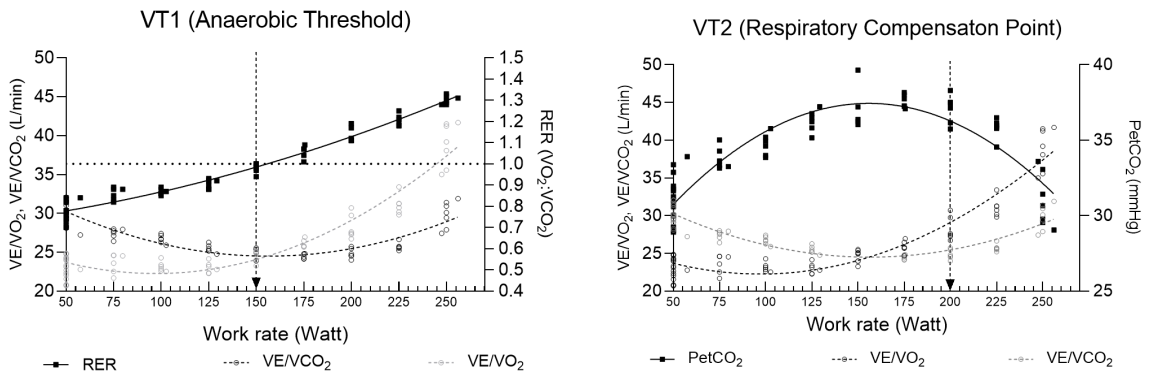

Figure 36. *Identification of ventilatory 1 and 2 thresholds during the exercise test.*

The Ventilatory Threshold (VT) refers to the exercise intensity during the test at which ventilation starts to increase at a faster rate than volume of oxygen utilization (VO_2). Evaluating the ventilatory profile during aerobic capacity exercise test from submaximal to maximum effort is commonly used to assess aerobic fitness, monitor, and prescribe exercise training, and to understand the exercise metabolic response in endurance athletes and clinical populations [142-144]. This non-invasive measurement of the ventilatory profile is commonly represented as two inflection points: the ventilatory threshold (VT) and the respiratory compensation point (RCP) [144-146]. For the prescription of exercise training, work intensities representing metabolic demands have been described as moderate (below ventilatory threshold or VT1) and vigorous intensity (above VT1) exercise. VT1 is described as the point at which pulmonary ventilation and carbon dioxide output begin to increase exponentially [142, 143]. For the severe exercise intensity domain, the respiratory compensation point (RCP, VT2) is considered the second break point in the ventilation profile described as the isocapnic buffering caused by chemoreceptor induced hyperventilation that reduces arterial CO_2 pressure below 40mmHg [147, 148]. The NASA Human Physiology, Performance, Protection, and Operations Laboratory and the Exercise Countermeasures Laboratory has developed a software algorithm that identifies VT1 and VT2 from these aerobic capacity exercise test (see Figure 36) [149].

Table 5. *Exercise intensity domains defined by traditional anchors of exercise prescription from an aerobic capacity exercise test [32].*

Anchor	**Moderate intensity**	**Vigorous intensity**	**Near maximal to maximal intensity; severe**
VO_2 max (% of max)	46–63%	64–90%	>90%
HRmax (% of max)	64–76%	77–95%	>96%
Ventilatory Threshold (VT)	Below VT1	Above VT1	Above VT2
Prescription of Exercise	Continuous 30 mins	High intensity—long bouts of 10–20 min with recovery	High intensity short bouts of 1, 2, 5 min intervals with recovery

Note: VO_2, volume of oxygen consumption; HR, heart rate; VT, ventilatory threshold; VT1, ventilatory threshold near anerobic threshold; VT2, ventilatory threshold near respiratory compensation point.

The aerobic capacity test can be used to individualize the exercise prescription for aerobic (cardiorespiratory) exercise training applying a crewmember's VO_2peak (%VO_2peak) and maximal heart rate (%HRmax) as described in Table 5.

Prior to the exercise test, a resting measure of electrocardiogram (ECG) and blood pressure are obtained at the end of 5 min resting in supine position and 3 min of seated rest. ECG, ratings of perceived exertion (RPE), and metabolic gas analysis are recorded over the duration the exercise testing. After testing, the ECG is recorded during the first 5 min of recovery and may be used to extend the recovery time frame, based upon clinical indications. One of two cycle exercise protocols is used for testing (either a "light" or "nominal" protocol), dependent on the crewmember's body weight. The light protocol is designed for those weighing < 65 kg, and the nominal protocol is used for an individual weighing > 65 kg. Nominal protocol may also be used for those crew weighing < 65 kg and who regularly perform cycle exercise training. The light protocol starts with a 45 W warm up for 3 min and increases workload by 15 W every min until volitional exhaustion, then is followed by a cool down at 45 W for 3 min. The nominal protocol starts with a 50 W, 3-min warm up and increases workload by 25 W every 1 min until volitional exhaustion, followed by a 3-min cool down at 50 W.

Test termination criteria include:

- Onset of symptoms consistent with angina pectoris
- Sustained ventricular tachycardia (defined as seven or more sequential complexes)
- Cardiac dysrhythmia not observed preflight accompanied with unusual symptoms of exertional intolerance
- Technical difficulties monitoring the ECG
- Unusual or severe shortness of breath (inconsistent with level of effort)
- Signs of poor perfusion, including pallor, cyanosis, or cold and clammy skin
- Volitional fatigue (as noted above, if the test results are deemed a submaximal effort the test is repeated)

Test rules and constraints include:

- If cardiac dysrhythmia causing symptoms or hemodynamic compromise noted, the crew surgeon will be notified, and the test will be postponed until approval to proceed is given
- Wearing workout clothing (shorts, t-shirts, sneakers) required
- No max aerobic exercise 24 hrs prior to testing; no aerobic exercise 8 hrs prior to testing
- No lower-body strength exercise 8 hrs prior to testing
- Limited caffeine intake to 1 cup (8 oz) of regular coffee or equivalent 8 hrs to 60 min before test
- No large meals 2 hrs prior to test. A light meal is permitted up to 30-60 min before test
- No alcohol, or nicotine 8 hrs prior to test
- Do not apply lotion to the torso on the day of testing (pretest)
- Each crewmember uses the PPFS to measure metabolic oxygen consumption and heart rate via ECG. As a redundancy, the crewmember must also don a Heart Rate Monitor
- Musculoskeletal injury that precludes crewmember from performing a maximal cycle ergometer effort
- Test Termination Criteria

ii) Isokinetic Muscle Strength and Endurance

This test is a medical requirement titled MEDB 5.3 Isokinetic Testing. The major objective is to provide pre- and postflight muscle strength and endurance measurements in select muscle

groups for the evaluation of in-flight countermeasures and postflight rehabilitation. Muscle performance testing is administered using a standard clinical isokinetic dynamometer on selected muscle groups. A standard protocol for warm-up prior to testing is followed for each muscle group. Testing is performed on the right limb, unless previous injury indicates that the left limb should be utilized for these assessments. The following test are used pre- and postflight:

- Concentric knee extension and flexion – Seated 60°/s 5 reps
- Endurance – 180°/s 21 reps
- Concentric ankle plantarflexion and dorsiflexion – Prone 30°/s 5 reps
- Eccentric ankle plantarflexion and dorsiflexion – Prone 30°/s 5 reps
- Concentric trunk extension and flexion – Standing 60°/s 5 reps

Test Termination Criteria include volitional fatigue, pain, lightheadedness, dizziness, or other symptoms of orthostatic hypotension and muscle strain or joint sprain.

iii) Functional Fitness Testing

Table 6. *Functional fitness testing, assessment, and hardware*

Test	Assessment	Major Muscle Group	Hardware
Sit and Reach	Flexibility	Lower back and hamstring	Acuflex I sit and reach box (Novel Products, Rockton, IL)
Cone Test	Agility	Whole body movement	Cones placed on corners of a 15 x 15 ft q area box
Push-ups	Muscle endurance	Upper limbs	No hardware
Pull-ups	Muscle strength and endurance	Upper limbs	Option to use squat rack, cable column, pullup/dip unloader machine, or straight bar hanging on the wall
Sliding Crunches	Muscle endurance	Abdominal	No hardware
Bench Press (1RM)	Strength	Upper limbs	Cybex Smith Press Machine (Cybex International, Medway, MA)
Leg Press (1RM)	Strength	Lower limbs	Cybex squat-press machine (Cybex International, Medway, MA)
Hand grip	Isometric strength	Hand and forearm	Grip A instrument (Takei and Co, Tokyo, Japan)
*Midthigh pull	Isometric strength	Whole body	FT700 Plyometric Power System (Fitness Technology, Skye, SA, Australia), Inflight: Resistive Exercise Device (ARED)

Note: 1RM, one repetition maximum; * indicates not a current measure of functional fitness testing.

Eight measures of functional fitness tests (MEDB 5.1) were developed as medical requirements that were supplemental to the muscle strength and endurance test (MEDB 5.3 Isokinetic Testing) to assess ISS crewmembers major muscle group fitness changes from pre- to postflight. Additionally, these tests evaluated the efficacy of in-flight exercise hardware [138]. Preflight testing occurs at 60-90 days before flight and 5-7 days after landing. Prior to the function

fitness testing, a 10-min warmup is completed on the cycle ergometer, treadmill, or elliptical machine which is then is followed by the measurement tests listed in Table 6.

Sit and Reach. This test evaluates the flexibility of the lower back and hamstring muscle groups. The crewmember removes their shoes, place feet against the footplate with hips and knees extended fully, and with one hand over the other reaches as far as possible holding briefly. The farthest distance after three trials is recorded.

Cone Agility. This test evaluates agility and ability to change directions quicky. The 1-5 movements are completed and total time to completion is recorded with a hand-held stopwatch. The best of three trials is recorded. Movements: 1) forward movement, 2) shuffle to the right, 3) backwards movement, 4) left shuffle, 5) figure 8 forward movement (see Figure 37).

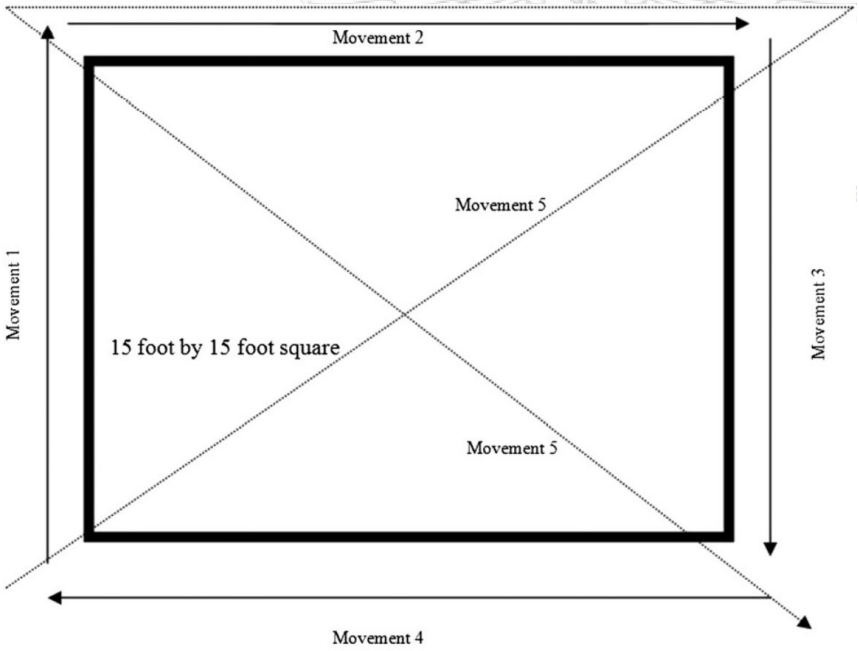

Figure 37. *Schematic setup and protocol of cone agility test.*

Push-ups. This test evaluates upper body muscle endurance. Crewmembers start with hands forward, under the shoulders, with head up and back parallel to the floor. The test starts when the body is lowered, and elbows are flexed at 90°, then move back to start position with elbows extended. Rest is allowed at the top position. The total number of pushups completed in 2 min is recorded.

Pull-ups. This test evaluates upper body muscle strength and endurance. Crewmembers are given a choice of using the squat rack, cable column, pullup/dip unloader machine, or straight bar hanging on the wall. Crew are also given the option to use either pronated or supinated grip. Full range of motion starts with the elbows extended and the repetition ends when the chin is above the bar. The total number of unbroken pull-ups completed is recorded. Hardware used and hand placement is recorded and will be similar at pre- and postflight assessments.

Sliding Crunches. This test evaluates muscle endurance of the abdominal muscles. The crewmember starts with back on floor and legs flexed to 90° and feet flat on ground, chin on chest, and arms at the sides of the body. Abdominal muscles are contracted as hands and arms remain on floor while sliding toward the heels of the feet. The total number of sliding crunches completed in 2 min is recorded.

Bench Press (1RM). This test evaluates upper body strength. One repetition maximum (1RM) test is completed after the warm-up. The warm-up begins with 2-3 sets at estimated 30% load for 10 repetitions. A 3-5 min rest period is allowed between trials. Weight is increased by 10-20% for each advanced set that decreases in reps until reaching 1 RM. Validation of 1 RM is

reached when crewmember fails to complete the next lift. This format has been modified from an earlier version of this test in which the 4-6 RM was determined and used to estimate the 1 RM.

Leg Press (1RM). This test evaluates lower body strength. 1 RM test is completed after the warm-up. The warm-up begins with 2-3 min of 10 repetitions at an estimated 50% of load. Weight is increased by 15-20%, then 5-10% with a decrease in repetitions until reaching 1 RM. Each set is followed by a 3-5-min rest. Validation of 1 RM occurs when crewmember fails to complete the next lift. This format has been modified from an earlier version in which the 4-6 RM was determined and used to estimate the 1 RM.

Hand Grip. This test evaluates hand strength. The instrument is held in the hand with the second joint of the forefinger flexed at 90°. Standing upright, the crewmember exerts full force while keeping the instrument at their side. Three trials are completed for each hand, and the maximal score for each had is combined as the total score. The hand grip test was added after Expedition 9.

Isometric midthigh pull. This test evaluates whole body strength. The instrument bar and mechanical stops are positioned approximately 2/3 down the length of the crewmember's thigh with the adjust turnbuckles set to the fix the bar position. The crewmember then places their hands at the standardized position on bar which are then secured using lifting straps over the hands. The warm-up is completed by performing three sub-maximal isometric pulls (one at ~50%, one at ~75%, and one at near max). Then a 1-min rest is provided prior to maximal attempts. Crew complete 3–5 maximal isometric force tests with 2-min recoveries between trials. Force plate transducers provide velocity, force, and power analysis that provide peak power, fatigue index, and total work performed. Fatigue index (%) = (repetition eliciting the highest power output [W]) – (repetition eliciting the lowest power output [W]) / (repetition eliciting the highest power output [W]). This test is currently in the process of being added into the functional fitness tests.

iv) Muscle Volume MRI and Ultrasound Testing

Loss of muscle mass is a major concern for long-duration spaceflight. Muscle mass is assessed using magnetic resonance imaging (MRI) and ultrasound methods. Pre- to postflight changes for leg muscle mass has been reported to be about ~20% loss, and this is at a rate of about -0.10/kg of lean body mass loss per week [51]. Recent studies have reported that leg muscle volume is reduced 7–15% at R+7 [52]. Recently, the utility of teleguided self-ultrasound scanning (Tele-SUS) has been developed by NASA to accurately monitor leg muscle size in astronauts during space flight [51]. Scott et al. [51] reported that after 168 days of spaceflight, 74 Tele-SUS sessions were performed and showed no significant differences between panoramic ultrasound images obtained by astronauts 7 days prior to landing and expert sonographer after flight or between change in muscle size assessed by ultrasound and MRI. These findings support current capabilities of ultrasound imaging to allow self-monitoring of muscle size with remote guidance.

k. Human Terrestrial Research (Ground Based Analogs)

Human physiology studies during space flight are difficult to perform due to the limited number of subjects available and multiple confounding factors, including variable adherence to prescribed countermeasures, inconsistent dietary practices, participation in other science experiments, and interference of specific mission task requirements [9, 150].

Bed rest has become an accepted and established model to study changes in physiologic function associated with space flight, including changes in VO_2peak, in a more controlled environment [151, 152]. In general, the reduction in VO_2peak as a result of bed rest is considered to result from the combined effects of reduced physical activity and removal of orthostatic stress [153]. In a direct comparison between responses after space flight and bed rest, Trappe et al. [9] reported that the decrease in estimated VO_2peak during supine cycle ergometry in four crewmembers 4 days after a 17-day mission (-10.4%) was comparable to that observed in eight subjects (-6.6%) 3 days after a 6° head-down tilt bed rest of the same duration (Figure 38).

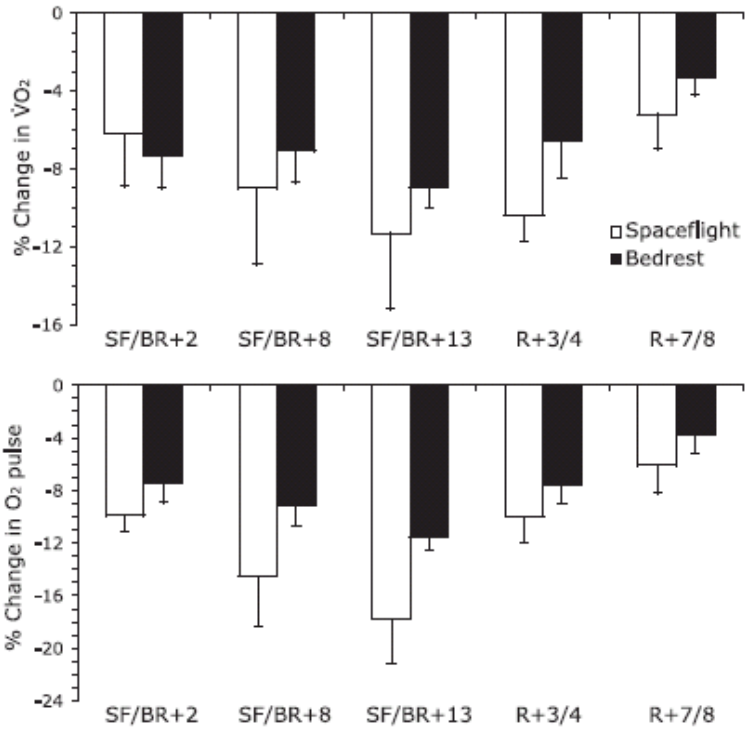

Figure 38. *Oxygen consumption (VO_2) and O_2 pulse changes occurring while exercising during and after both spaceflight and bed rest. Spaceflight data were collected at 85% of the preflight maximal workload, while bed rest data were collected at maximal effort [9].*

Several ground-based paradigms have been used to emulate the effects of microgravity unloading on human skeletal muscle, including complete horizontal or 6° head-down-tilt bed rest, dry immersion, and unilateral upper- and lower-limb unloading with or without joint immobilization. In general, skeletal muscle responses to unloading have been similar in these models. Although no perfect simulation of crew activities and the microgravity environment can be adequately achieved, Adams and colleagues have suggested that bed rest is an appropriate model of space flight for studying skeletal muscle physiologic adaptations and countermeasures [154]. Absent from human analog studies are the unique operational and psychological stressors associated with space flight that exacerbate the physiological changes resulting from muscle unloading [155, 156]. Finally, in anticipation of a future, long-duration human presence on the moon, Cavanagh et al. have developed a lunar bed rest model that incorporates standing and sitting with axial skeleton loading of 1/6 bodyweight [157].

I. Factors that Affect Aerobic Capacity

Several factors can affect the magnitude of change in VO_2peak during bed rest. These include the bed rest duration, pre-bed rest fitness level, gender, and whether a countermeasure is performed. The effectiveness of specific exercise countermeasures will be reviewed in greater detail in a later section. A visual representation of the oxygen transport cascade in Figure 38 shows the interaction of pulmonary and peripheral circulation and muscle metabolism.

i) Cardiac Output and Circulation

Cardiac output (Qc) is the product of heart rate (HR) and stroke volume (SV). In ambulatory subjects, it has been postulated that the primary determinant of VO_2peak is maximal Q_c. Although the debate continues in scientific journals to this day, many have argued that the capacity of the muscular system to increase vascular conductance and oxygen consumption is greater than the ability of the human heart to pump blood [158-162]. The integrated roles of the cardiovascular, respiratory, and skeletal muscle systems to support oxygen transport and metabolism during exercise are depicted in Figure 39. Supporting the view that maximal Q_c is a primary limiting factor after bed rest, the reduction in Q_c in five male subjects following 21 days of bed rest (−26%) was similar to the reduction in VO_2peak (−26%) [163]. Similarly, using radionuclide imaging in 12 middle-aged men, Hung et al. [164] observed a 23% decrease in maximal Q_c and a 17% decrease in VO_2peak following 10 days of bed rest. However, this relationship between the decrease in maximal Q_c and lower VO_2peak after bed rest does not appear to remain as the duration of the bed rest is extended. For instance, Capelli et al. [165] reported that decrease in Q_c after 42 and 90 days of bed rest was not significantly different than that measured after 14 days of bed rest, suggesting that peripheral factors at the level of the working muscle were responsible for further decrements in VO_2peak. In contrast, Ferretti et al. [166] reported that maximal Q_c was reduced to a greater extent than VO_2peak (−31% and −17%, respectively) after 42 days of bed rest. However, a non-traditional peak cycle protocol was used in this study, with each incremental stage lasting 5 min in duration but separated by a 5-min rest period. Therefore, it is possible that this protocol did reflect the true change in VO_2peak.

In general, maximal HR has been observed to be unchanged or to slightly increase after short and long-duration periods of bed rest [152, 165, 167], and therefore is not likely a contributing factor to a lower maximal Q_c. Maximal HR was unchanged following 24 hrs of bed rest [168], but in a separate study it was observed to increase during both supine (5.7%) and upright (5.9%) cycle exercise following a 10-day bed rest [153]. The effects of exercise countermeasures on maximal HR after bed rest are inconsistent. Recently, maximal HR was unchanged in control subjects following 30 days of bed rest and was decreased when an exercise countermeasure was employed [31], but it was increased after bed rest with and without an exercise countermeasure in another study [169].

Reduced SV is the primary contributor to the decrease in maximal Q_c. Hung et al. [164] reported that after 10 days of bed rest, the reduction in Q_c was solely the result of a 28% reduction in exercise SV. Similarly, maximal oxygen pulse, considered to be an index of SV, was reduced after 10 [94] and 17 days [9] of bed rest during supine ergometry, and comparable responses were noted in four astronauts following a space flight of the same duration [9]. Ferretti et al. [166] reported that the 31% decrease in maximal Q_c following 42 days of bed rest was due solely to a 31% reduction in maximal SV because maximal HR was unchanged. Resting and submaximal exercise SV also were reduced during long-duration bed rest [170-172] and space flight [173].

Although exercise SV is consistently reduced with bed rest, studies reporting the effects of bed rest on cardiac function are not consistent. Convertino et al. [37] showed an increase in ejection fraction and suggested that ventricular performance is maintained while venous return and cardiac filling may be impaired. In contrast, more recent data suggest that left ventricular impairment occurs with bed rest due to changes in the distensibility of cardiac tissue [174-176] and a reduction in cardiac diastolic function [177]. Cardiac atrophy, measured using MRI, appears to occur by 14 days of bed rest, likely contributing to reduced cardiac distensibility and smaller SV for a given filling pressure. Left ventricular mass decreased in men by 5, 8, and 16% after 2, 6, and 12 weeks of bed rest, respectively [178]. Additionally, left ventricular end-diastolic volume

decreased by 14% after 2 weeks of bed rest, but changed only minimally thereafter. Similar observations were made in women after 60 days of bed rest [179]. There is evidence that the addition of an exercise countermeasure during bed rest prevents or mitigates losses in cardiac function [180, 181].

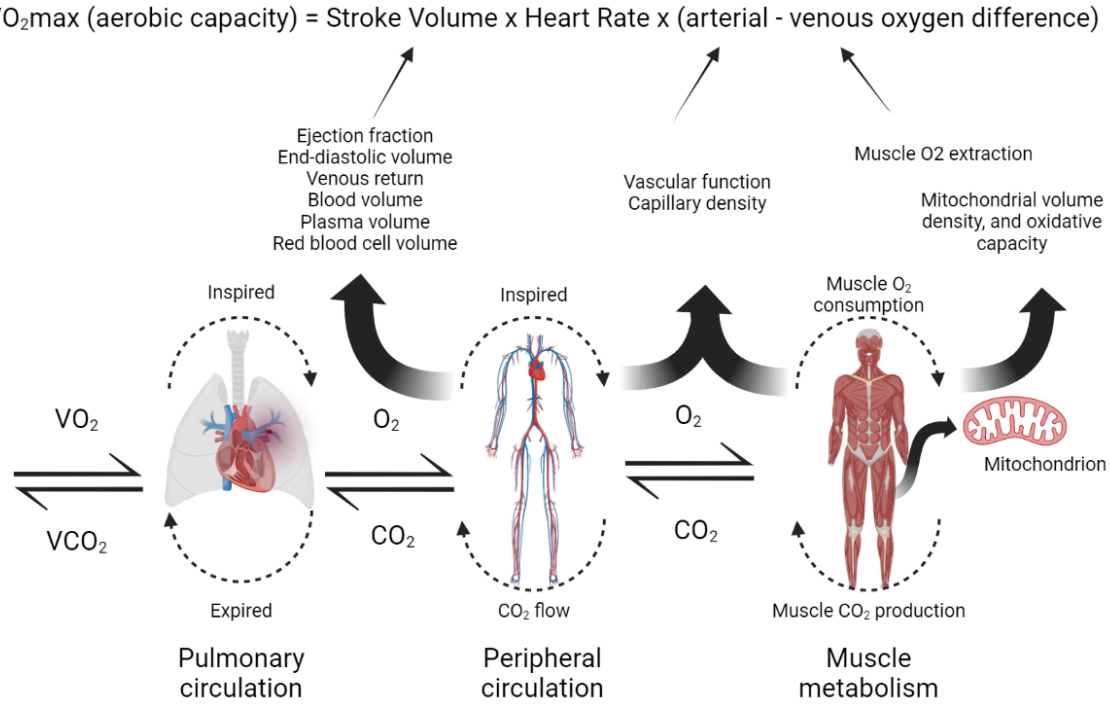

Figure 39. *Depiction of oxygen transport and the interaction between respiratory, cardiovascular, skeletal muscle systems to support oxidative metabolism during exercise. VO_2, volume of oxygen; VCO_2, volume of carbon dioxide.*

ii) Blood Volume

Previous investigations consistently have demonstrated that plasma volume is rapidly reduced during exposure to space flight and bed rest, with the majority of the initial loss occurring within 1-2 days [182]. Plasma volume has been observed to be decreased in as little as 6 hrs, reaching a 10% loss in 24 hrs, and equaling approximately 12% by the third day of bed rest [168]. Greenleaf et al. [183] have suggested that the loss of plasma volume is progressive through 60 to 80 days of bed rest. The time course of the decrease in plasma volume is similar to the decrease in exercise capacity (Figure 40), and the mean loss of plasma volume across studies has been reported to account for approximately 70% of the variability in the mean decrease in VO_2peak following up to 30 days of bed rest [37]. Reduced circulating plasma volume may negatively affect exercise SV, the delivery of oxygen and nutrients to working muscle, and the removal of metabolic waste products. Thus, preservation of plasma volume has been suggested to be an important factor in the maintenance of exercise capacity during bed rest and may be even more important during upright than supine exercise because of the addition of gravitational stress.

Maintenance of plasma volume alone is likely not the only determinant of exercise capacity preservation, especially following longer duration bed rest studies. Blomqvist et al. [184] provided sufficient amounts of saline through infusion to restore the central venous pressure to the pre-bed rest levels following a 24 hr bed rest. Although this procedure was not protective of orthostatic tolerance, it did abolish the loss of upright VO_2peak [184]. In contrast, Stremel et al. [169] maintained plasma volume (−8%, NS) in subjects during 14 days of bed rest by employing two daily 30-min bouts of supine cycle exercise at 68% of pre-bed rest VO_2peak, but supine VO_2peak (−9%) and submaximal exercise responses were not maintained. In addition, subjects who performed an isometric exercise regimen during the same study experienced loss of plasma volume that was similar to the non-exercise control subjects (−15% vs. −10%), but the decrease in VO_2peak was not as great (−5%) in the isometric exercise group [169]. In a separate study, restoration of plasma volume at the end of a 16-day bed rest following an intense exercise bout did not successfully maintain VO_2peak [185].

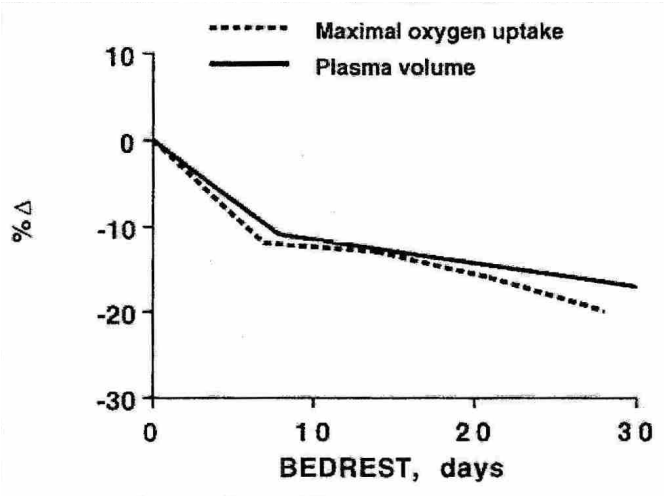

Figure 40. *Decreased maximal oxygen consumption in subjects who perform no countermeasures appears to parallel losses of plasma volume up to 30 days of bed rest [37].*

When reporting the results of plasma volume and VO_2peak for individual subjects, the relationship between these two outcomes may not be as strong as when comparing mean results for a group of subjects. In general, there appears to be more variability in the plasma volume response to bed rest than in the decrease in VO_2peak. Additionally, as the duration of the bed rest period increases, the strength of the relationship appears to decrease. Following 14 days of bed rest, in control subjects and subjects participating in a countermeasure employing a reverse pressure gradient garment, the decrease in VO_2peak was significantly related to the loss of plasma volume (r^2=0.56) [186]. Recently, in a study of male control and exercise countermeasure subjects, the change in plasma volume from pre-bed rest accounted for only 24% of the variance in upright VO_2peak after 30 days of bed rest [187]. In a companion study utilizing female control and exercise subjects, there was no significant change in plasma volume in either the control or countermeasure subjects, although only the control subjects experienced a significant loss of VO_2peak. The lack of change in plasma volume in both the female control and exercise subjects appears to support previous observation that VO_2peak is not strongly related to the change in plasma volume with bed rest [188].

iii) Venous Return

Decreased venous return may be the result of an increase in lower body venous compliance and reduced plasma volume that has been commonly observed after bed rest [37]. Although multiple vascular factors contribute to limb compliance, changes in muscle mass and tonicity associated with bed rest may contribute to increase venous pooling when the mechanical obstruction to venous stretching and accumulation of blood is reduced. Following 30 days of bed rest, calf compliance was increased (2.4%) concomitant with a decrease in muscle volume (−5%) [189]. In this bed rest study, changes in muscle cross-sectional area explained approximately

50% of the variability in the change in calf compliance. When subjects are exercising, it is recognized that cardiac filling pressure, SV, and Q_c are supported by the expulsion of blood from the active muscles by the "muscle pump," but these mechanisms will be of little importance in non-active muscles and other compliant regions of the circulation.

Blood may pool in other areas of the body after bed rest, including the splanchnic region, which would affect venous return during exercise. Savilov et al. [190] used radioisotope tracers to measure translocation of blood during LBNP, an orthostatic stressor. Subjects with low orthostatic tolerance displayed a marked increase in blood pooling in the abdomen during LBNP, with reflective decreases in blood distribution to the head and chest. Subjects with relatively better LBNP tolerance had less extreme responses [190]. Similarly, Fischer et al. [191] reported that splanchnic blood flow was higher at each level of LBNP following just 4 hrs of bed rest, and this was associated with an increased HR and reduced volumes [191].

The reduction in vasoconstrictive reserve that Convertino and Cooke suggest as a factor in orthostatic intolerance after bed rest and space flight may also contribute to reduced exercise capacity [192]. Following 16 days of bed rest, elevated vasoconstriction was evident at rest in response to reduced plasma and SVs [193], and maximal vascular resistance was unchanged but was achieved at a lower level of orthostatic stress induced by a graded lower body negative pressure protocol [194]. An inability to vasoconstrict—particularly in the venous system, which contains 70% of the total blood volume of a resting subject—impairs the ability to compensate for decreased blood and plasma volume, especially when coupled with orthostatic stress, to maintain venous return and SV during exercise.

Linked to this, changes in sympathetic nervous system response to exercise may be important to VO_2peak with regard to the appropriate distribution of blood flow. Specifically, there is an inverse relationship between norepinephrine concentrations and splanchnic blood flow. Rowell calculated, for example, that regional vasoconstriction in the splanchnic organs, kidneys, and skin can provide an additional 600 ml of O_2 per minute at maximal exercise in normal ambulatory subjects [160]. Elevated levels of circulating norepinephrine may be an important adaptation to reduced blood volume to defend muscle blood flow and restrict flow to the splanchnic region and other inactive tissues [195]. Sympathetic nervous system activity and catecholamine levels in resting subjects have been reported to be either unchanged or decreased following bed rest, and elevated HR in resting subjects has been ascribed to reduced vagal control [196]. Following 3 days of bed rest, the norepinephrine levels during submaximal exercise tended to be higher, and the norepinephrine threshold was lower in endurance athletes following bed rest, but these alterations were not evident in sedentary subjects [197]. There was, however, no difference in maximal norepinephrine concentrations or epinephrine responses in either group. In contrast, following 16 days of bed rest, Engelke and Convertino [195] reported that plasma norepinephrine concentrations were 64% greater at peak exercise although peak heart rate was only 5% higher. However, no changes in epinephrine were reported during rest or maximal exercise.

iv) Decreased Red Blood Cell Mass

Red cell mass has been reported to be decreased in as little as 7 days of bed rest [198], although most consistent results are observed at bed rest day 14 [152]. Additionally, red cell mass may continue to decline for a short period during the recovery from bed rest [8, 152, 199]. Convertino et al. [186] reported that red cell volume was decreased by 11% during 14 days of bed rest, independent of whether the subject performed no countermeasures or participated in a protocol to simulate the effects of orthostatic stress. While exercise during bed rest may prevent

the loss of red cell mass, exercise that is too intense has the potential to cause red cell destruction [152]. Similarly, after prolonged space flight of up to 6 months, it has been reported that destruction of red blood cells occurs [12].

The correlation between the change in red cell mass and the change in VO_2peak is low in short- and moderate-duration bed rest studies [37]. In general, hematocrit does not change during bed rest, suggesting that the oxygen carrying capacity per unit of blood is unchanged [37, 186]. However, as red cell mass continues to decline with longer bed rest, albeit at a slower rate, the total oxygen delivery capacity of the blood is reduced at rest, during submaximal exercise, and further impaired at maximal exercise when maximal Q_c also is reduced [151, 166, 170]. Capelli et al. [165] reported that hemoglobin concentration was decreased by 9% after 42 days of bed rest, which—along with the decrease in Q_c—was reflected in a 34% decrease in total oxygen delivery. However, arterial saturation of hemoglobin was unchanged during bed rest [165, 166].

v) Arteriovenous Oxygen Difference

Maximal systemic oxygen extraction, assumed to be at the level of the working muscle, does not appear to be affected by short-duration bed rest. There was little change in arteriovenous oxygen difference in middle-aged men following 10 days of bed rest [164], and it was unchanged after 21 days of bed rest in five male subjects [163]. In general, the maximal extraction of oxygen from the systemic circulation does not appear to be specifically affected by bed rest, but oxygen extraction during submaximal exercise appears to be increased in compensation for lower hemoglobin concentration during longer durations of bed rest [200]. However, it is not clear from these data whether blood flow is appropriately directed to working muscle or whether the extraction at the level of muscle itself is maintained.

Delivery of oxygen to the muscle has been suggested to be impaired after bed rest. Resting leg blood flow [201] and peak vascular conductance, which has been associated with VO_2 max in ambulatory subjects [202], are reduced following bed rest [185, 201, 203-207]. The reduction in vascular conductance was associated with a decreased resistance to fatigue of the calf muscle, but when peak vascular conductance was restored with a maximal bout of exercise at the end of bed rest, VO_2peak was not similarly protected [185]. However, peak vascular conductance was associated with VO_2peak before and after bed rest, suggesting that protection of peripheral mechanisms associated with the oxygen utilization in the muscle are not effective unless central cardiac effects are restored [185]. Additionally, Hikida et al. [208] reported a 37% decrease in the capillary-to-fiber ratio of the soleus following 30 days of bed rest, although Ferretti et al. [200] observed no change in either capillary density or capillary-fiber ratio in the vastus lateralis.

vi) Orthostatic Tolerance and Cerebral Perfusion

The influence of gravity on work performance is apparent when comparing results from supine versus upright exercise capacity after equal durations of simulated microgravity. After short-duration bed rest, VO_2peak decreased 2-2.5 times more during upright exercise compared to supine exercise [153, 198]. After 10 days of bed rest in middle-aged men, the reduction in VO_2peak was 15% in the upright posture but was not significantly different when subjects were tested in the supine posture [153]. Submaximal exercise responses are similarly affected; at 115 W, HR was elevated by 4% above pre-bed rest values when subjects performed supine ergometry but was increased by 8% when the exercise was performed upright [153]. Exercise in the upright posture is associated with a greater reduction in SV and Q_c than supine exercise. Saltin et al. [161] reported that both resting and exercise SVs were reduced to a greater extent when subjects

were upright (Rest: −24%, Exercise: −35%) than when the subjects were supine (Rest: −17%, Exercise: −23%).

Exercise alone prevents the loss of VO_2peak when pre- and post-bed rest tests are performed in the supine posture. A potential relationship between the preservation of orthostatic tolerance and exercise performance by implementation of a single countermeasure would be an attractive feature to NASA. During recent studies utilizing exercise and LBNP as an orthostatic stressor, the countermeasure subjects maintained VO_2peak [2, 187] and experienced smaller bed rest-induced changes in cardiovascular responses during orthostatic stress [209] and attenuated orthostatic intolerance [210]. Using these data to specifically link orthostatic tolerance and exercise capacity is weakened when relying upon these data sets alone because the countermeasure is a combination of exercise and orthostatic stress.

Inadequate cerebral perfusion during post-bed rest exercise might also impair exercise performance, particularly when performed against an orthostatic stress. Prior to 30 days of bed rest, the majority of subjects terminated graded exercise tests due to general fatigue and shortness of breath [187]. After bed rest, half of the control subjects who performed no countermeasures reported lightheadedness or loss of balance as the primary reasons for test termination. In contrast, fatigue and shortness of breath remained the predominant symptoms at test termination after bed rest in a group of subjects who performed an exercise countermeasure which maintained VO_2peak [187].

The regulation of blood flow to the brain during rest to standing and during exercise may be influenced by the reduced exercise capacity and changes in central command. The strength of voluntary maximal muscle contraction is reduced to a greater extent (−36%) than muscle tension (−24%) which is electrically-evoked. The difference between these two muscle tensions, termed the force deficit, increased by 40% during bed rest [211]. If muscle performance is inhibited in this manner following a period of unloading, VO_2peak and Q_c, according to the “oxygen pull” model, consequently, also would be reduced [159, 170].

vii) Duration

In general, there is a rapid decline in VO_2peak with the first few days of bed rest and a more gradual loss thereafter [153]. Nixon et al. [168] reported a decrease in estimated VO_2peak tested during upright cycle ergometry of 22% (Pre: 36.4±2.4; Post: 28.5±2.0 $ml \cdot kg^{-1} \cdot min^{-1}$) following only 24 h of 5° head-down tilt bed rest. During bed rest periods up to 30 days, the average decrease in VO_2peak occurs at a rate of 0.8–0.9 $\% \cdot d^{-1}$ [37, 167, 212]. However, if VO_2peak continued to decrease linearly in this fashion, the predicted decrease in VO_2peak would be 42% by 60 days and 72% by 90 days of bed rest, which overestimates the reduction in VO_2peak in longer duration bedrest studies and would reach zero (resulting in death) within 122 days. However, a recent meta-analysis of bedrest data found that VO_2peak declines at a rate of 0.008 L/day (Figure 41) [41]. Current exercise countermeasures (standard and SPRINT exercise prescriptions [52]) maintain fitness for the group crew population on average compared to the control group with no exercise, but they aret still not fully protective and show a slight reduction slightly above the 95% UCI of the estimated loss in VO_2peak (see Figure 41).

Capelli et al. [165] proposed an alternative model based upon measurements during bed rest studies ranging from 14 to 90 days. Subjects in their studies experienced a decrease in VO_2peak of 14% on day 14, 16% on day 42, and 32% on day 90 of bed rest [165]. The authors speculated that the initial rapid reduction in VO_2peak was due to decreased maximal Q_c and circulating hemoglobin levels, while the later slow progressive component was related to muscle atrophy and impairment in peripheral gas exchange. Similarly, Greenleaf et al. [213] observed the greatest rate of decrease in VO_2peak in the first week of bed rest.

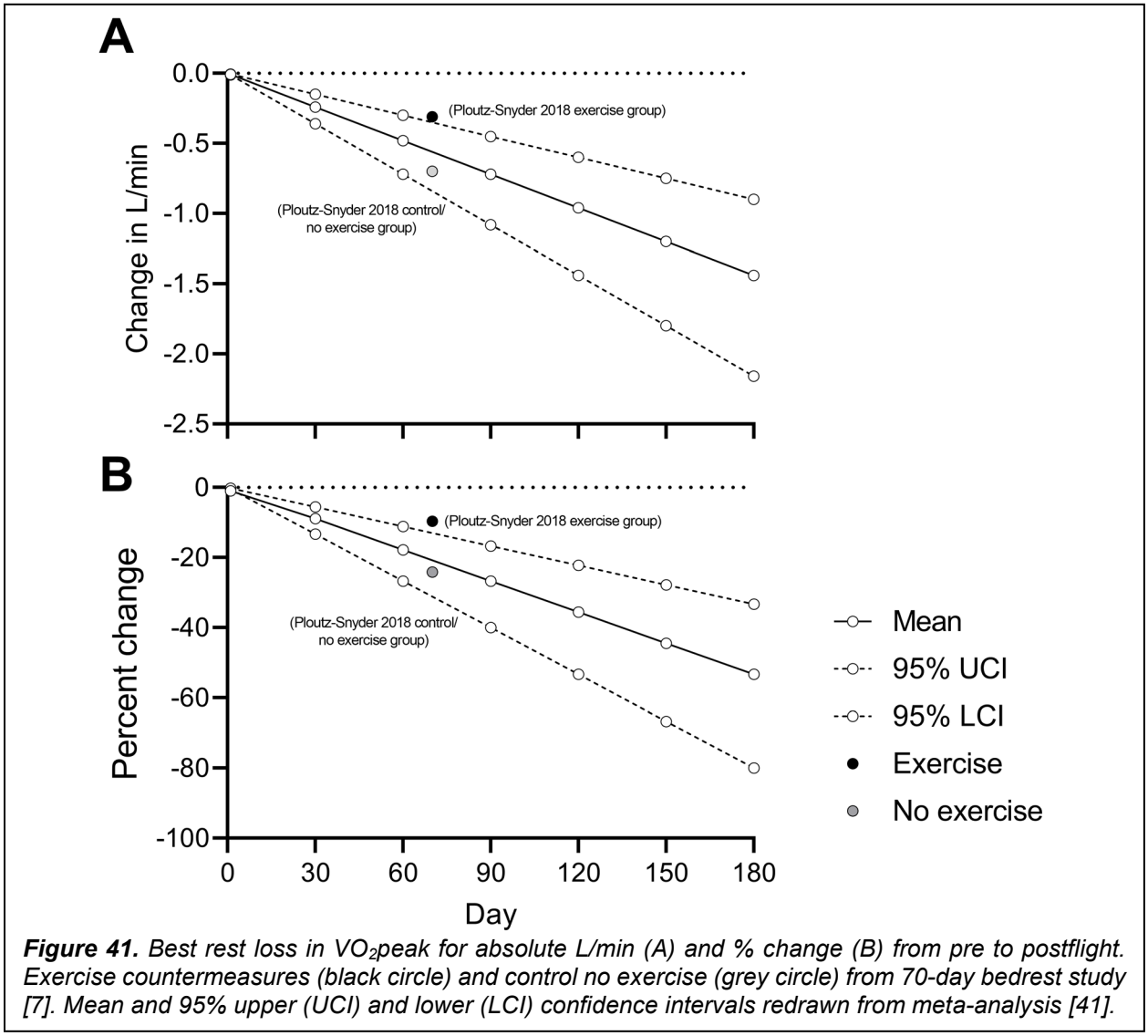

Figure 41. *Best rest loss in VO_2peak for absolute L/min (A) and % change (B) from pre to postflight. Exercise countermeasures (black circle) and control no exercise (grey circle) from 70-day bedrest study [7]. Mean and 95% upper (UCI) and lower (LCI) confidence intervals redrawn from meta-analysis [41].*

Decreased VO_2peak [214] and delayed oxygen kinetics [215] during the first 1–2 weeks of bed rest generally are associated with decreased circulating blood volume. More recent research has also shown that only five days of bed rest significantly effects cardiac geometry and function [216]. During longer simulated microgravity exposures, structural changes in the myocardium [175, 178, 179] and the vasculature [217] may increasingly impair exercise capacity as the duration of bed rest increases. For example, bed rest data suggest that left ventricular impairment occurs due to changes in the distensibility of cardiac tissue [174-176] and a reduction in cardiac diastolic function [177]. However, the addition of an exercise countermeasure prevented cardiovascular

deconditioning after 5 weeks of bed rest [180, 181]. Additionally, negative metabolic adaptations to simulated microgravity, such as reduced citrate synthase activity in skeletal muscle, become apparent after 4 weeks of unloading [208, 218]. Longer durations of bed rest are associated with decreased muscle mass, strength, and endurance which would be expected to impair aerobic exercise performance and decrease the efficacy of the muscle pump to protect venous return [219].

Table 7 *Short and prolonged time course changes to circulatory, neurovestibular, skeletal muscle, and bone systems and behavioral health that have major impacts on aerobic capacity and muscle strength.*

		Duration of flight				**Duration of Post-flight**			
Physiological Time Course	Launch	24–48h	1 mo	>1mo	Landing	24–48	1 wk	1–2 mo	>1 yr
Circulation	−10% fluid volume, fluid redistribution to torso and head[10, 11]		Gradual decrease in erythropoietin secretion and increase red blood cell destruction[12]		Orthostatic intolerance (hypotension) [10, 11]	Return to normal fluid distribution[10, 11]			
Neurovestibular/sensorimotor	Space motion sickness[44] and disorientation[49]				Motion sickness, spatial orientation and balance impaired[49]		Sit to stand, recovery from fall, tandem walk returns to normal by R+7		
Skeletal Muscle		Gradual −20% muscle mass, −0.10/kg rate of lean body mass loss per week, 0.10 kg[51]			Muscle soreness and tightness[51]		*Muscle volume reduced 7–15% at R+7[52]		
Bone Density		Rate of bone loss per month, 0.8–1.5%[53]					6+ mo bone density reduced by 6% spine, 8% femoral neck, 8% proximal femur[54] *Bisphosphonates + exercise[55]		
Behavioral	Fatigue, sleep debt, isolation, emotional stress. Increase in cortisol by 10% at days 10–20[54]								
Aerobic Capacity		Est. rate of loss per day, 0.8% or peak VO_2 of 0.008L/ min[41]				*Reduced by 10–15% at R+1[47, 52]		*Returned to preflight at R+30[47, 52]	
			Decrease of 17% in-flight by day 15[47]				*Reduced by~6% at R+10[47]		
Muscle Strength		−6%/wk rate of loss per week for knee extensor strength 5–6%[34]			Muscle soreness and tightness[34, 51]		*Reduced by ~6–17% in knee strength R+5[52, 59]	*Reduced by 5–10% at R+30[52, 59]	
			−25% leg extension strength after 28 day[34]						

The impact of flight on VO_2peak and muscle strength and endurance is dependent on duration of spaceflight. Moreover, changes of other organ systems (circulation, sensorimotor, skeletal muscle mass, bone density) and behavioral health will affect the time course changes in VO_2peak and muscle strength and endurance (Table 7).

viii) Pre-Bed Rest Fitness

Individuals with higher fitness levels have a greater potential for reduction in VO_2peak. Taylor et al. [220] and Saltin et al. [163] were the first to report that men with a higher VO_2peak had a greater absolute reduction after bed rest than those with lower fitness. Subsequent studies confirmed this hypothesis [221, 222]. Greenleaf and Kozlowski [223] observed that this relationship was strongest when subjects performed peak cycle ergometer tests in the supine position, while the relationship was not strong during upright treadmill testing. Conversely, data from the 30-day WISE twin bedrest studies found a significant correlation between pre-bedrest fitness and loss in VO_2peak measured during upright treadmill exercise in both male and female non-exercising subjects [31, 187]. With respect to gender, Convertino et al. [221] reported that there was a significant relationship between initial VO_2peak and VO_2peak measured after 10 days of bed rest in middle-aged and young men (r=-0.84 and r=-0.78, respectively), but not in either middle-aged or young women (r=-0.25 and r=-0.38, respectively). The gender difference might be a consequence of the lower pre-bed rest VO_2peak values and the shorter bed rest duration. Overall, the data support the contention that those who have higher initial fitness levels are more prone to losses in VO_2peak, but they maintained a higher absolute fitness level compared to their less fit counterparts.

ix) Recovery after Bed Rest

The time course of the recovery of VO_2peak following bed rest is dependent on the bed rest duration and the degree of deconditioning. Some improvement in exercise responses is apparent within a few days of reambulation. Similar to the data reported for space flight, this is largely associated with a recovery of plasma volume, particularly apparent during submaximal exercise. For example, the HR response to treadmill walking was significantly elevated on the first day of reambulation following a recent 60-day bedrest study but was not different than pre-bedrest 2 days later, even though VO_2peak was still significantly lower (-21%) than the pre-bedrest value [3]. Similarly, there was some recovery of submaximal HR during supine ergometry (150 W) 3 days after a 17-day bed rest period compared to the last in-bed rest test, even though VO_2peak was still depressed compared to pre-bedrest (-7%) [9]. Although the time course for recovery of post-bedrest plasma volume has not been well characterized, preliminary data from 90-day bedrest studies suggest that plasma volume on average is restored after 3 to 4 days of ambulatory recovery in non-exercise control subjects (Dr. Steven Platts, personal communication).

Recovery of VO_2peak normally occurs within 2-4 weeks after bedrest lasting 30 days or longer [224]. Recovery is much faster after shorter duration of bedrest. For example, VO_2peak was 6.6% below pre-bedrest levels after only 3 days of reambulation and was ~3% below pre-bedrest levels after 1 week of reambulation [224]. A similar amount of recovery (50%) was observed in four crewmembers after a space flight of the same duration (R+8: -5.2%, R+3: -10.4%) [9]. Saltin et al. [163] reported that the aerobic capacity of 3 of 5 subjects who participated in 21 days of bedrest was restored within 10 to 14 days after resuming normal activities. Additionally, the fitter subjects appear to return to their pre-bed rest fitness levels more slowly than their less fit counterparts, although the previously more highly fit subjects are likely to perform better at all time points than if they had been previously unfit [163].

Following 60-day and 90-day bedrest studies conducted by NASA Johnson Space Center, VO_2peak estimated from submaximal exercise tests was improved during the recovery period (from R+2 to R+11) in most subjects but remained 10% lower than pre-bedrest VO_2peak in 5 of the 9 subjects (Dr. Don Hagan, unpublished observations). All of the bedrest subjects participated

in a daily 1 hr program of supervised ambulation and exercise during the post-bedrest period. The program consisted of 10–15 min of walking as well as calisthenics to strengthen the muscles of the trunk, upper body, and legs. The primary objective of the reconditioning plan was to restore the functional mobility and capacity to perform activities of daily living in preparation for release from bedrest. Since the protocol was not targeted specifically at increasing VO_2peak, it is not surprising that recovery of VO_2peak was incomplete. Similarly, Sundblad et al. and Spaak et al. observed that submaximal heart rate was elevated at 12 and 15 days, respectively, after 42-days of bed rest, but returned to pre-bedrest levels when tested 32 days after bed rest [171, 172].

x) Submaximal Exercise Response

Changes in VO2peak, HR response to submaximal exercise, and lactate threshold may occur independently of changes in VO_2max [225], which could signal a potential for earlier onset fatigue and the impaired ability to perform sustained tasks. Aerobic deconditioning after bedrest is evident by higher HR, ventilation, respiratory exchange ratio, and rating of perceived exertion during submaximal exercise [2, 152, 167, 168, 186, 187, 197, 198, 226-228]. Of these, elevated HR during submaximal exercise is the most prominent feature of bedrest-induced deconditioning. Submaximal exercise HR was increased following 24 hrs of bedrest by approximately 20 beats·min^{-1}, which Nixon et al. [168] noted was similar to the increase observed in Apollo and Skylab astronauts following space flight. During 17 days of bedrest, submaximal HR at a workload of 150 W was significantly increased by the eighth day of bed rest and remained elevated throughout the first post-bedrest exercise test [9]. Bedrest studies that incorporate an aerobic exercise countermeasure show mixed results, where the effectiveness of the countermeasure appears to be related to the prescribed exercise intensity. Submaximal exercise HR was increased in almost every stage during supine ergometry following 14 days of bed rest, whether subjects performed a moderate intensity exercise countermeasure or not [169]. However, when subjects perform a countermeasure which preserves VO_2peak, submaximal HR is unchanged from pre-bed rest levels [2, 31, 38, 187].

Elevated submaximal exercise HR after bed rest likely is a compensatory mechanism to maintain Q_c when SV is decreased. Following 20 days of bed rest, submaximal Q_c was not different during upright exercise, although HR was increased and SV was reduced in subjects performing no countermeasures [38]. A 3-day bed rest study investigated the cardiovascular responses at rest and during submaximal exercise in sedentary and endurance trained subjects after 3 days of bed rest. At rest, HR increased, and SV was reduced in both sedentary and endurance trained subjects; whereas SV was reduced, and HR was elevated only in the sedentary subjects during submaximal exercise. The fact that no change was observed in endurance athletes might have been because the submaximal workloads (up to 150 W) represented a proportionally lower percentage of their maximal exercise capacity (Peak W: Control = 188, Endurance = 270)) [197]. Other changes in the submaximal exercise response associated with detraining include elevated ventilation and respiratory exchange ratio [229].

Lactate threshold may be the most important predictor for exercise performance and the ability to perform sustained tasks as may be required during EVA or future exploration missions. To date, only one study has measured the effects of bed rest on lactate threshold and showed that the decrease in lactate threshold was far greater than the decrease in VO_2peak (25% vs. 7%) following 10 day of bed rest [230]. Others have measured blood lactate during submaximal exercise and have shown higher concentration post-bed rest compared to pre-bed rest [163, 197, 230-232]. The effect of bed rest on the lactate threshold may be more apparent in more highly trained subjects who also experience a decrease in the norepinephrine response threshold during graded exercise after 3 days of bed rest [197]. The impaired ability of skeletal muscle to utilize

aerobic pathways after bed rest for energy utilization might be inferred from the loss of aerobic pathway enzymes [208] and reduced glucose transporter content [233] or to reduced or inappropriate distribution of blood flow, as has been observed in animal models during exercise [234].

xi) Heat Stress, Sweat Rates, and Thermoregulation

Human expenditure of energy results in the generation of heat. The body heat generated by normal activities, and particularly by exercise, triggers homeostatic regulatory mechanisms with the goal of maintaining body core temperature within its relatively narrow, safe physiologic range by means of vasoregulation and diaphoresis. The weightless environment of space flight may impair heat dissipation by reducing evaporative and conductive heat exchange. Microgravity and space flight possibly perturb the body's thermoregulatory mechanisms through altering the work efficiency, metabolic rate, or circadian rhythms of heat production. Additionally, human space travelers are often not well hydrated, have a 10–15% decrease in intravascular fluid (plasma) volume, and may lose both their preflight muscular and cardiovascular fitness levels as well as their thermoregulatory capabilities. As a result, they may become less heat-acclimated or may acquire an altered thermal sensitivity [35, 235, 236]

Alterations in thermoregulation in association with space flight could have significant impacts on a variety of space flight-associated activities including exercise (as a countermeasure to muscle atrophy, cardiac deconditioning, and bone loss), EVA, and vehicle landing and egress. EVA suits and launch and entry or advanced crew escape suits (ACES) worn by ISS and Shuttle crewmembers are designed to provide an impermeable barrier between the wearer and the external environment. To compensate for lack of heat exchange through the fabrics of these suits, the EVA suit provides both liquid (conductive) and air (convective) cooling, as a liquid cooling garment is worn under the ACES in addition to a hose connection to forced orbiter cabin air. Thus, crewmembers with altered thermoregulatory capabilities are at even greater risk should failure of the cooling systems of these garments occur [237]. Manifestations of altered thermoregulation include increased heart rate and body temperature during exercise, decreased work capacity and endurance, decreased postflight orthostatic tolerance, decreased cognitive ability, and a delay in recovery of exercise capacity and endurance after flight [35].

Thermoregulation has been studied in association with both space flight [35, 238] and 6˚ head-down-tilt bed rest [238-240]. To date, there have been no direct measurements of heat balance during in-flight exercise sessions. In the only space flight study, submaximal exercise and thermoregulatory responses were recorded before flight and at 5 days after landing in two crewmembers who completed a 115-day mission [35]. Normal heart rates were observed for both crewmembers during supine exercise for 20 min each at 20% and 65% of VO_{2max} [35]. However, during postflight (5 days after landing) testing, exercise was voluntarily discontinued after only 8-9 min of supine exercise at the 65% of VO_2peak level for the two crewmembers when both experienced difficulty in maintaining pedaling frequency and complained of leg fatigue and their heart rates exceeded the highest recorded preflight levels. Both crewmembers exhibited a more rapid increase in body core temperature during the shorter postflight exercise session than during the preflight session (Figure 42). It was concluded that heat production was not altered, but that impairment of heat dissipation due to altered vasodilatory and sweating responses were responsible for the increased rate of rise in the core body temperature.

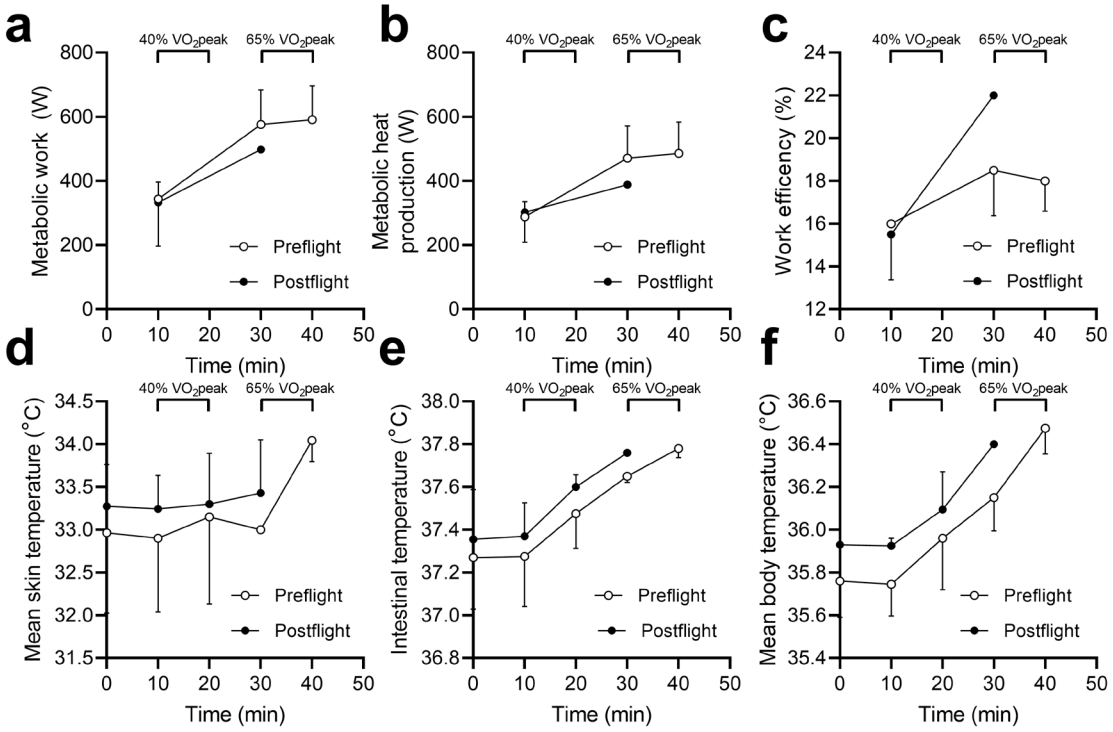

Figure 42. *Body temperature and thermoregulation during submaximal exercise after 115-day space flight drawn from [35]. Sample size: n=2 astronauts at pre- and postflight return day 5. Means ±SD.*

Physical work capacity after bed rest and space flight may be further reduced by impaired body temperature regulation during rest and exercise that, in turn, potentially leads to heat strain and injury. With regard to space flight, the combined effects of plasma volume loss and loss of heat acclimation may result in excessive heat strain for crewmembers wearing protective garments during launch and landing [241]. During a nominal landing (STS-90, April 1998) prior to exit from the Space Shuttle, intestinal temperature (core temperature) was significantly elevated in four crewmembers wearing the required Launch and Entry Suit (LES), despite the use of a liquid cooling garment [242]. In the event of an emergency egress from the Shuttle, crewmembers would be disconnected from the thermoelectric cooling unit supplying the liquid cooling garment in order to exit the vehicle, and they would then be required to ambulate to a safe distance. This activity would be completed fully suited and may require an effort exceeding 70% of the crewmember's preflight VO_2peak [70]. The combined thermal load of the protective garment and the elevated metabolic rate during egress would likely rapidly increase core temperature.

Changes in thermoregulatory control may be impacted during and after space flight. Leach et al. [243] reported that evaporative water loss was reduced by an average of 11% in nine Skylab crewmembers during their in-flight exercise as compared to preflight. The authors suggested that the sweating responses may have been reduced in the microgravity environment through the formation of a film of sweat on the skin's surface, resulting from reduced sweat dripping, which then impaired air flow across the skin and impeded sweat evaporation. Further, reduced gravity would have limited spontaneous convection, in which air rises or falls due to differences in density, and low air flow in the cabin of space vehicles during space flight may limit heat loss capacity [35, 235, 236, 244].

Fortney et al. [35] observed that the thermoregulatory mechanisms were impaired in two crewmembers when performing exercise following long-duration space flight (115 days) onboard the Mir space station. Both crewmembers had mildly elevated core temperature at rest and after 20 min of exercise at 40% VO_2peak and had a delayed onset of sweating rate response and skin vasodilation. Neither crewmember was allowed to complete the second exercise stage post-flight (20 min at 65% preflight VO_2peak) because the flight surgeon terminated the test "due to an excessive rise in HR" [35]. Despite the shortened exercise time, both crewmembers had a core temperature at the end of the post-flight exercise bout similar to their core temperatures at the end of the entire exercise protocol during preflight testing. Both crewmembers exhibited lower skin blood flow and sweating rate responses that may have contributed to this elevated core temperature.

Impaired thermoregulation at rest and during exercise is evident after bed rest. Crandall et al. [245] passively heated subjects with a warm water-perfused suit before and after 15 days of bed rest. After bed rest, these subjects had a reduced forearm blood flow and vascular conductance before and during whole body heating [245]. Michikami et al. [246], using similar techniques, also observed an increase in the threshold temperature and decreased sensitivity of the vascular conductance and sweating response following 14 days of bed rest. A higher core temperature has been observed after bed rest during submaximal exercise in both warm and temperate conditions [8, 197, 247-249]. The elevated post-bed rest core temperature during exercise was ascribed to a decreased ability to increase skin blood flow and impaired sweating responses [8, 248, 250] (Figure 43). However, the performance of an exercise countermeasure during bed rest has been shown to prevent these thermoregulatory adaptations and preserve VO_2peak [251].

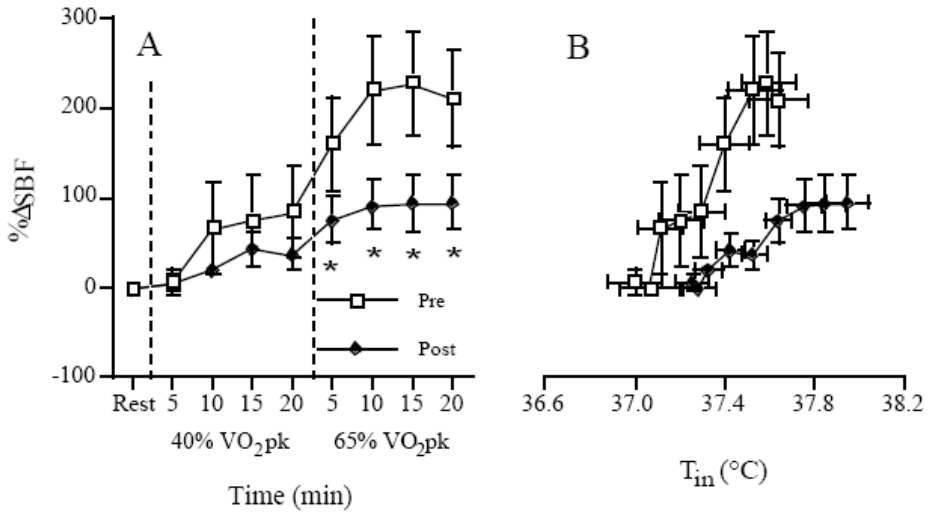

***Figure 43**. (A)The percent change in skin blood flow (%ΔSBF) and (B) increasing core temperature during submaximal exercise is reduced after bed rest. The onset of the vasodilatory response was delayed, and the slope of the response tended to be reduced [8, 9].*

m. Factors that Affect Muscle Mass, Strength & Endurance

Bed rest unloading causes a significant loss of body nitrogen and lean body mass [6, 20, 252]. A reduction in the size or volume of the ambulatory muscles accounts for most of the decrease in lean body mass after bed rest [6, 23, 26, 253]. Horizontal and 6° head-down-tilt bed rest protocols of durations ranging from 1–17 weeks have resulted in significant reductions in lower-limb muscle mass as measured by DXA (mass) or MRI (CSA or volume). Decreases in muscle volume after bed rest are paralleled by decreases in muscle strength and endurance (Table 8), as evidenced by significant reductions in angle-specific torque [254], isokinetic muscle strength [6, 255], and fatigability [256]. Similar losses in muscle volume, strength, and endurance have been observed after unilateral lower-limb suspension [255, 257, 258]. Dry immersion, a whole-body-unloading paradigm with the added advantage of mimicking the reduced proprioceptive input encountered during space flight, also brings about reductions in muscle volume, strength, endurance, electrical activity, and tone [259-265].

Table 8. *Changes in leg lean mass (LLM), knee extension (ext.) strength, and ankle extension strength in bed rest studies of various durations.*

Study	BR duration (d)	Δ LLM, CSA, or volume (%)	Δ Knee ext. strength (%)	Δ Ankle ext. strength (%)
Alkner 2004	29	−10.0 (vol)		
	89	−18.0 (vol)	−60.0	
Bamman 1998	14		−14.5	
Berry 1993	30	−11.0 (CSA)		
Dudley 1989	30		−24	
Ferrando 1996	14	−3.9 (mass)		
Gogia 1988	35		−19.0	−24.4
Kawakami 2001	20	−7.8 (CSA)	−10.9	
Kortebein 2007	10	−6.3 (mass)	−15.6	
LeBlanc 1988	35			−26
LeBlanc 1992	7		−14.7	−7.2
	35		−25.2	−12.5
	119	−11.9 (vol)*	−30.7	−19.9
Mulder 2009	60	−13.5 (CSA)	−21.3	−24.9
Paddon-Jones 2004	28		−17.8	
Suzuki 1996	20	−10.6 (vol)	−23.6	
Trappe 2007	29	−16.8 (vol)		
	60	−21 (vol)	−33.7	−42.1
*measured by dual-photon absorptiometry; vol = volume, CSA = cross-sectional area. [6, 16-28]				
Suzuki 1996	20	−10.6 (vol)	−23.6	
Trappe 2007	29	−16.8 (vol)		
	60	−21 (vol)	−33.7	−42.1
*measured by dual-photon absorptiometry; vol = volume, CSA = cross-sectional area. [6, 16-28]				

Decreases in muscle volume with unloading are rapid and persistent. LeBlanc et al. [6] reported significant losses of ~6% in both the quadriceps and soleus/gastrocnemius of young men after 14 days of bed rest. Although attenuated in rate, the losses in these muscles continued throughout 17 weeks of bed rest with final losses of 16–18% and 30% in the knee and ankle extensors, respectively [6]. Findings from short- [6, 16, 18-20, 22, 23, 26-28] and long-duration bed rest studies [6, 16, 25, 28] corroborate this pattern of rapid initial losses followed by reduced but continued decrements. Thus, although absolute decreases in muscle mass are greater with longer periods of unloading, rates of loss are higher in the first several weeks of disuse.

Young women appear to lose muscle volume at similar if not slightly faster rates as men during bed rest inactivity. In two separate investigations utilizing the same MRI methodology, young men and women rapidly reduced quadriceps (–10% and –17%) and triceps surae (–16% and –18%) muscle volume after 29-day bed rest [28, 266]. After 89-day (men) and 57-day (women) bed rest in these same subjects, quadriceps (–18% and –21%) and triceps surae (–29% and –29%) muscle volume were further reduced although at attenuated rates, particularly in the quadriceps in men [28, 266]. Thus, the gastrocnemius/soleus muscles are more vulnerable to unloading-induced losses than the quadriceps.

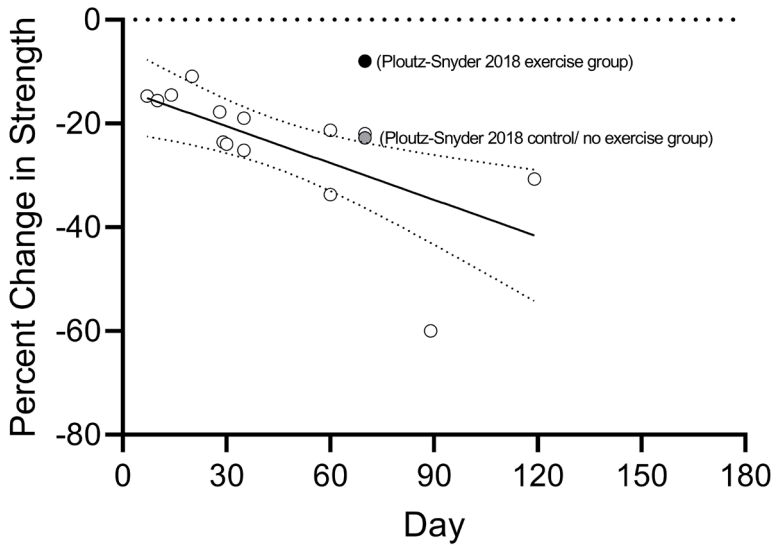

Figure 44. *Time course of percent change in knee extension strength for bed rest studies with no exercise (open circles, references in Table 7). Exercise countermeasures prevents the loss in strength; however, they are not fully protective. In comparison to the exercise group (black circle) and no exercise control group (grey circle) 70-day bed rest study [7].*

Strength decreases during unloading are 1.5–3 times that of muscle mass (percent change) [6, 16, 22, 23, 25, 27, 28] (Figure 44). Bamman and colleagues [17] observed losses of 18, 17, and 13% in concentric, eccentric, and isometric plantar flexor peak torque, respectively, after 14 days of bed rest; Akima and co-investigators [267] observed a 16% decrease in knee extensor isometric torque after 20 days of bed rest. Although not specifically reported, subjects in an 89-day bed rest trial [268] experienced significant reductions in isokinetic torque in the lower body, with the greatest losses in the knee extensors (−35%). This study also used isotonic testing (1RM), and mean losses ranging from −6 to −37% were observed [268]. Additionally, reductions

in adductor, abductor, and leg press strength were on the order of ~25–30% [268]. In an earlier 90-day bed rest trial, LeBlanc and colleagues [6] observed losses of 31% in knee extension strength and 15% in knee flexion strength. Similar to changes in muscle mass, unloading-induced strength losses are often greater in the ankle plantar flexors than in the knee extensors [21, 25, 28], although this is not always the case [6, 16]. Compiling bed rest studies and pre- to postflight changes for knee extension strength are reported in Figure 44, showing a linear relationship between change in strength and duration of bedrest. For comparison the exercise and no exercise control group for a 70-day bed rest study are shown in the Figure 44 and demonstrates the no exercise is similar as the trend line whereas the exercise group shows some level of protection [7]. Exercise does not fully protect against the loss in knee strength but does offer some countermeasure against the spaceflight deconditioning.

Few studies have reported changes in the ab/adductor or the flexor/extensor muscles of the hip. Shackelford et al. [268] reported that isotonic strength decreased by about 25% in the adductors, but only a 6% decrease in the hip flexors was demonstrated after 17 weeks of bed rest. After 55 days of bed rest, Berg et al. [269] reported that a 22% reduction in isometric hip extension occurred, although the extensor muscles in the gluteal region decreased in volume by only 2%. The authors reported no explanation for this discrepancy between the proportion of reduced strength relative to the loss of mass and stated that no previous studies in the literature had made these concurrent strength/volume measurements in the hip musculature.

i) Neural influences

As enhanced neural function plays a significant role in the increased muscle strength associated with early adaptation to resistance exercise training [270], the reverse is also true as decreases in neural function contribute to the reduction in strength observed with unloading. The neural maladaptation include decreased electrically-evoked maximal force [271], reduced maximal integrated electromyography [255, 272], increased submaximal electromyography [272], neuromuscular junction dysfunction [273], and reduced specific tension [272]. After 23 days of unilateral lower limb suspension (ULLS), de Boer et al. [274] observed an increased electromechanical delay and reduced rate of torque development during maximal voluntary contraction of the knee extensors, an effect also observed previously [17]. However, the central activation ratio and normalized electromyography root mean square were unchanged [274]. Fifty-six day bed rest also caused no change in root mean square during maximal voluntary contraction of the knee extensors but elicited decreases in median firing frequency and fiber conduction velocity [275]. Fiber conduction velocity of the vastus lateralis and tibialis anterior was also reduced in both single motor units and whole muscle during submaximal contractions after 14 days of bed rest [276].

ii) Muscle Protein Synthesis, Breakdown, and Cell Signaling

The primary mechanism of muscle loss during unloading is a reduction in muscle protein synthesis (MPS) [23, 277-280], which is significantly correlated with the decrease in muscle mass during bed rest [20, 281]. Activation of the mammalian target of rapamycin (mTOR) pathway, a key regulator of translation initiation, is required to stimulate muscle protein synthesis [282]. It is thus not surprising that bed rest causes a diminished phosphorylative response of mTOR and its downstream targets, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) to anabolic stimuli (e.g., essential amino acids) [283].

Muscle protein breakdown (MPB) is more difficult to measure, and several studies have shown no changes with bed rest [20, 252, 280]. However, recent evidence suggests that MPB

plays an important role in the atrophic response to unloading, particularly during the first days of disuse. Tesch et al. [284] used a microdialysis technique to sample 3-methylhistidine concentration in the vastus lateralis before and after ULLS and found a 44% increase in muscle proteolysis after only 72 hrs of unloading. In their brief review, Attaix et al. [285] noted a number of recent studies that demonstrate a strong interconnectedness between the regulation of synthetic and proteolytic pathways during disuse; however, none of this work is in humans. Marimuthu et al. [286] argue that, in light of observed increases in ubiquitin-protein conjugates [287] and 3-methylhistidine [284], an early and transient increase in muscle protein breakdown, is partially responsible for disuse-induced muscle atrophy.

iii) Fiber Changes and Enzyme Activity

At the structural level, the loss of muscle volume in disuse models correlates with a significant decrease in CSA of both Type I and Type II myofibers [17, 208, 258, 288-291]. In general, Type II myofibers seem to be more likely to atrophy than do Type I myofibers during short-term unloading, with no significant myofiber type shifting being observed [17, 288, 292], although alterations in total muscle MHC protein isoform expression have been reported [293]. However, in the prolonged 84-day bed rest, Type I fibers in the vastus lateralis atrophied to a greater degree (−15%) than did Type IIa fibers (−8%), and a shift to a faster fiber type occurred in both the vastus lateralis (Type I to Type IIa and Type IIa to Type IIx) and the soleus (increased hybrid fibers) at the expense of Type I fibers [294, 295]. More recently, a 35-day bed rest caused large, but relatively uniform. CSA decreases of 31%, 21%, and 28% in Type I, Type IIa, and Type IIx vastus lateralis myofibers, respectively [296].

Immobilization by limb casting does not seem to reduce the relative proportions of muscle-specific proteins, such as carbonic anhydrase II and myoglobin, over that predicted by the overall decrease in muscle protein synthesis [297]. In contrast, experimental evidence suggests that the specific activity of muscle enzymes involved in oxidative metabolism, such as pyruvate dehydrogenase, is decreased by cast immobilization [298]. A similar reduction in the activity of citrate synthase, but not phosphofructokinase, has been detected in the vastus lateralis, indicating a significant impairment of the oxidative capacity in this muscle after ULLS [218]. The differences observed between cast immobilization and ULLS or bed rest protocols may reflect the former being a better model of muscle atrophy induced by hypokinesia and the latter two being better models of muscle atrophy induced by muscle hypodynamia. The latter situation more closely resembles the actual conditions experienced by crewmembers during space flight, namely removal of mechanical loading without a reduction in limb mobility.

iv) Insulin Resistance

Additional research findings exist that relate peripherally to this risk description that should remain associated with it. First, secondary to the decrease in muscle mass associated with mechanical unloading is an increased susceptibility to insulin resistance and glucose intolerance. Second, crewmembers chronically exposed to the microgravity environment may develop impaired body temperature regulation during rest and exercise that may lead to heat strain and injury. These are discussed more fully in the following paragraphs.

Bed rest studies [299, 300] have shown an increased insulin response to glucose tolerance tests. Plasma insulin levels have increased up to four-fold compared to those of control subjects, and blood glucose levels exceeded those of the controls 2 hr after glucose loading. Similarly, Stuart et al. reported impaired glucose tolerance and a greater than 40% increase in both fasting plasma insulin and the insulin response to a glucose challenge. Suppression of hepatic glucose

production by insulin was unchanged after bed rest, indicating that insulin sensitivity was reduced only in skeletal muscle and not in the liver [301]. After a 28-day bed rest, Brooks et al. showed an increase in fasting insulin levels in an amino acid-supplemented group; whereas amino acid supplementation with resistance exercise decreased insulin values during bed rest. These changes were negatively correlated to changes in midthigh muscle area and were positively associated with whole body fat mass [302].

v) Nutrition

Reduced energy intake during unloading greatly exacerbates lean tissue loss. Biolo et al. [303] studied young men during a 14-day bed rest with either eucaloric energy intake or a 20% hypocaloric diet similar to the energy deficits reported in space flight. During eucaloric bed rest, subjects lost a small amount of lean mass (300 g), while hypocaloric bed rest provoked an almost four-fold greater decrease (1100 g). This magnified loss in the hypocaloric condition was facilitated by greater whole body net protein catabolism (i.e., MPS < MPB) in the post-absorptive state [303]. Excess energy intake during bed rest unloading is also deleterious and accelerates lean mass loss via increased systemic inflammation [304]. Thus, it appears that targeted eucaloric intake is key to the maintenance of lean mass during mechanical unloading.

Protein intake also plays a key role in the protection of lean mass during disuse. A simple 7-day bed rest study demonstrated that low protein intake (0.6 g/kg/d) causes a reduction in whole body protein synthesis, while higher intakes (1.0 g/kg/d) during bed rest prevent this [252]. Nutrition during unloading is further discussed below in the Countermeasures section.

Several studies have examined the effectiveness of supplemental protein or essential amino acids as a countermeasure to unloading-induced adaptations in skeletal muscle; some have shown positive effects (e.g., attenuated lean mass loss), while others have not [28, 302, 305]. In light of suggestions that the Recommended Daily Allowance (RDA) of 0.8 g protein/kg/d is too low , Stein and Blanc have argued that the positive outcomes for supplemental protein or essential amino acids in bed rest are simply due to the provision of adequate total protein (e.g., 0.8 g/kg/d in control diet + 0.6 g/kg/d supplementation = 1.4 g/kg/d) and not to an effect of the supplement *per se* [306]. This view is supported by the fact that the studies with negative findings for supplemental protein/essential amino acids all provided a control diet of ≥ 1.0 g protein/kg/d. Despite this seemingly simple conclusion to the supplemental protein/essential amino acids question, other research suggests that provision of adequate, or ideally, optimal, protein intake is more complex than just g/kg/d and is also modified by age [307]. In a 7-day study of ambulatory young adults, Mamerow et al. [308] examined the effects of protein distribution across the three daily meals on muscle protein synthesis. Because of their young age and ambulatory status, these subjects could be assumed to be the least responsive to this sort of subtle intervention. Regardless, provision of protein in an evenly distributed pattern of 30 g/meal (90 g protein/d) elicited greater muscle protein synthesis than consuming the same 90 g/d in a 10/15/65 g split across breakfast, lunch, and dinner [308]. This practical intervention is based on mechanistic work which shows that muscle protein synthesis is maximally stimulated by 10–15 g of essential amino acids (~30 g whole protein) and, perhaps more importantly, by ≥ 3 g leucine [309-311]. The influence of age on the acute, meal-based muscle protein synthetic response is seen in work by Katsanos et al. [312, 313] which showed that, in contrast to young adults, older adults had an attenuated muscle protein synthetic response to a small serving of essential amino acids containing only 1.7 g leucine, but the response was normalized to that of the young controls with the addition of 1.1 g leucine (2.8 g leucine total). Similarly, Rieu et al. [314] demonstrated that a mixed meal with 30 g protein but only modest leucine content (2.4 g leucine) when supplemented with additional leucine (~3.9 g; total leucine = 6.3 g) increased muscle protein synthesis in older

adults while the same meal with supplementary alanine failed to do so. Together, these data underscore the importance of adequate essential amino acid/leucine intake at each meal to maximally stimulate muscle protein synthesis, particularly in older adults [315-317]. Given that these studies demonstrated an anabolic resistance in older, ambulatory individuals, it is likely that careful, meal-based protein/leucine intake is necessary to optimize skeletal muscle outcomes during unloading when even young adults quickly become resistant to the anabolic effects of essential amino acids [278, 318].

Only one study has evaluated the combined effects of resistance exercise and nutrition countermeasures during unloading. Brooks et al. [302] showed that a 15 g essential amino acid supplement (with 2.8 g leucine) provided either before or after daily resistance exercise training during the 28-day bed rest was largely able to protect skeletal muscle mass and function, while the supplement without exercise was significantly less effective [302]. Unfortunately, the study did not include a resistance training-only control group to facilitate an understanding of any additive effect that the nutritional supplement may have provided [302]. A final piece of ground-based evidence salient to the optimization of skeletal muscle outcomes via nutrition involves the provision of supplemental protein immediately prior to sleep. Res et al. [319] demonstrated that 40 g of protein ingested just before bedtime was effectively digested and absorbed, increased muscle protein synthesis, and improved net balance overnight.

Nutritional inadequacies (e.g., insufficient protein or energy intake) can exacerbate muscle losses during unloading, while protein/essential amino acid supplementation affords only partial, or in some cases no, protection for muscle when employed alone. However, efforts to optimize the anabolic potential of dietary protein within the context of adequate total energy and protein intake should include further work examining the effects of protein distribution across meals and of supplementation prior to prolonged periods of fasting such as before sleep; as a potent anabolic agent, the essential amino acid leucine should be a primary candidate for supplementation. Little or no evidence exists to describe the synergistic effects of supplemented/optimized protein intake and exercise countermeasures during unloading, nor conversely, does work exist that elucidates the impact of sub-optimal nutrition on otherwise effective exercise countermeasures.

vi) Aging

Age also modulates the loss of muscle strength and reduced aerobic capacity. Cross-sectional studies have reported a decline in peak $\dot{V}O_2$ of 5% to 10% per decade in untrained individuals that is likely due to age-associated decrease in vigorous physical activity and skeletal muscle mass [40, 43, 320-323]. Felg. et al [322] examined aerobic capacity from the Baltimore Longitudinal Study of Aging and reported that decline in peak $\dot{V}O_2$ was observed in each decade (21–87 years) in both sexes; however, the rate of decline per decade was not linear. Aerobic capacity was between 3% to 6% per 10 years in the 20s and 30s, then increased to >20% per 10 years in the 70s and beyond. Moreover, the rate of decline for each decade was larger in men than in women from the 40s onward. Our preliminary ISS dataset in relation to normative data show crew are slightly above average within their age group variability both pre- and post-flight (Figure 45).

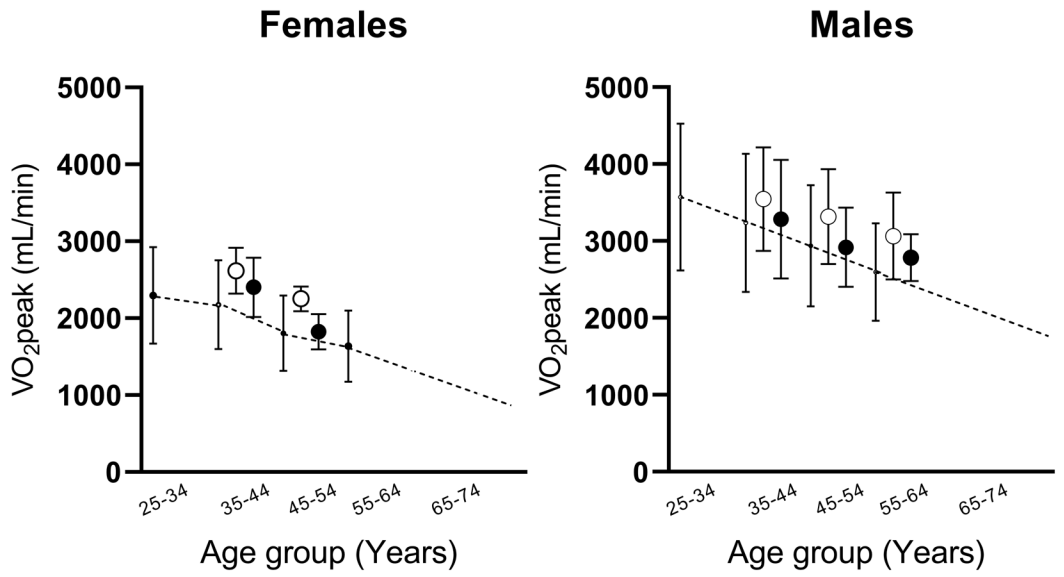

Figure 45. *Preliminary ISS VO_2peak pre- (open circle) to postflight (filled circle) in relation to age related normative data (dashed lines). Normative data (dashed line) from [40]. ISS dataset pre- to postflight (L-3/1mo to R+3d), mission duration 185±48 days, n=47 (male 36/ females 11), Mean ± SD*

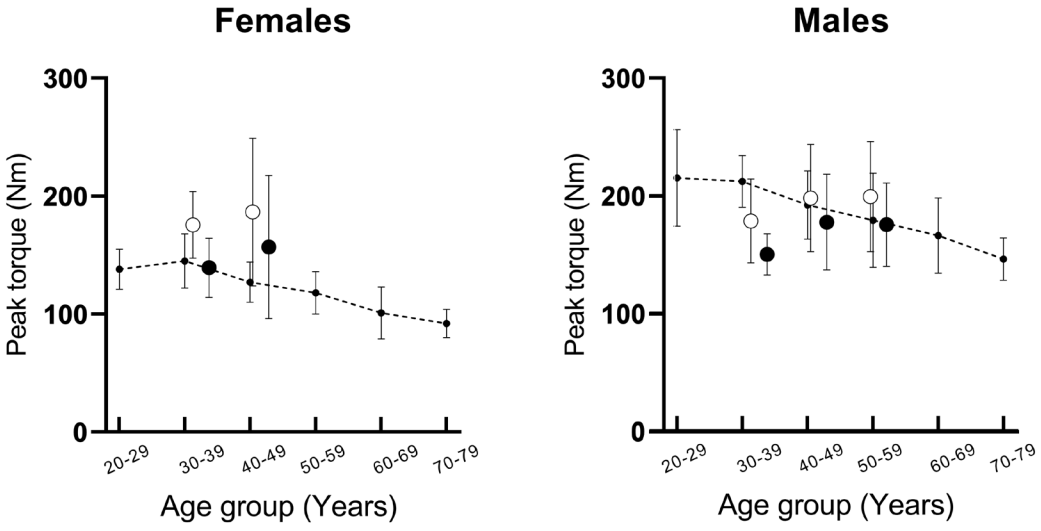

Figure 46. *ISS Isokinetic peak knee extension pre- (open circle) to post flight (filled circle) in relation to age related normative data (dashed lines). Normative data (dashed line) from [43]. ISS dataset pre- to postflight (L-3/1mo to R+5d), mission duration 174±37 days, n=98 (male 76/ female 22), Mean ± SD*

In the general population, muscle strength is significantly related to age, height, and sex [43]. Normative (1 g) data report that rate of change of in knee extensor and flexors with age is about 2% or more per year [321]. Although muscle loss in the young (30–35 y) during inactivity is considerable (e.g., 300–600 g leg lean mass in 14 days) [20, 303], older adults (67 y) lose lean mass at more than double the rate (950 g leg lean mass in 10 days) of their younger counterparts [23]. Not surprisingly, these reductions are accompanied by significant decrements in muscle strength and power [324]. Similar to young adults, bed rest-induced muscle atrophy in older adults is mechanistically driven by a reduction in post-prandial muscle protein synthesis, mTOR signaling, and amino acid transporter content [283]. No published data exist for unloading-induced alterations in muscle mass and metabolism in middle-aged individuals who would be representative of typical crewmember age. However, combined with the insidious onset of sarcopenia around the age of 40 [325], it is likely that middle-age (40–55 y, astronaut age) is associated with an accelerated rate of inactivity-induced alterations in muscle compared to young adults. We report in our preliminary ISS dataset of peak strength for knee extension in relation to normative data and show crew are slightly above average for females; however, the 30-39 age male group are under their age group variability (Figure 46).

n. The Female Astronaut

Current knowledge of space flight-induced changes in human physiology and performance has primarily been based on studies in a predominantly male astronaut population. The first women recruited for the U.S. space program occurred during the early 1960s. Thirteen women, named the “Mercury 13”, successfully passed the intensive battery of testing to become astronauts for the Mercury space program. However, due to lack of funding and concerns on the ability and safety of women to perform as astronauts, no one from the Mercury 13 flew in space [326]. In 1963, the first woman to travel to space was Soviet Cosmonaut Valentina Tereshkova, and 20 years later NASA sent Dr. Sally Ride to space as the first U.S. female mission specialist on the historic Challenger Mission STS-7. Nearly 60 years after sending the first male astronaut to the lunar surface, NASA has committed to the goal of sending the first female astronaut to the Moon as part of the Artemis Lunar Missions [327]. While this goal highlights the continued need for diversity within NASA’s space program, our understanding on the physiological implications that space flight has on the female body, and how it may differ from the male body, is limited. A greater understanding of the sex effects on adaptation to space flight will help determine and develop appropriate countermeasures for minimizing risk and maintaining health in all astronauts.

As of 2022, a total of 360 people have been selected to become NASA astronauts, 61 (17%) of whom are females (Table 9). While female astronauts have performed far fewer space flight missions (12.7% compared to 87.3% of male space flight missions), the average flight duration for female astronauts is nearly double that of their male counterparts. Similarly, female astronauts have greater cumulative days in space compared to male astronauts. Early space programs (i.e., Mercury, Gemini, and Apollo) were shorter in duration (<2 weeks) and included only male astronauts. The relatively short-duration of space flight missions during these early programs contributes to the difference in average flight duration between males and females. However, with longer duration ISS missions and future exploration class missions to the Lunar surface and Mars, understanding how extended microgravity and partial gravity exposure impacts all astronauts is imperative.

Table 9. *NASA Astronaut Demographics Grouped by Sex*

NASA Astronaut Demographics		Male		Female	
Astronauts (n, %)		299 (83.1%)		61 (16.9%)	
Astronauts with ≥1 space flight (n, %)		270 (84.4%)		50 (15.6%)	
Space Flight Missions (n, %)		955 (87.3%)		139 (12.7%)	
		Mean±SD	Range	Mean±SD	Range
Age at selection (yr)		34.4±3.7	(25–45)	32.5±3.5	(26–46)
Age at first mission (yr)		40.7±4.6	(32–58)	37.8±4.2	(32–55)
Time from selection to first space flight (days)		2288±1156	(765–6968)	2185±794	(765–3782)
Flight Duration (days)		25.7±49.7	(0–355)	40.6±68.7	(0–329)
Cumulative Duration in Space (days)		67.6±94.8	(0–534)	102.4±127.2	(0–667)
Height (in)		70.2±2.2	(64–76)	65.8±2.3	(60–72)
Body Weight (lbs)	*PRE First Mission*	175.9±19.2	(129–243)	136.4±19.9	(98–187)
	POST First Mission	173.5±18.9	(130–231)	132.8±19.9	(94–186)
	PRE All Missions	177.0±19.5	(108–246)	135.8±19.3	(97–187)
	POST All Missions	176.9±19.9	(130–244)	133.9±19.6	(91–187)

The innate physiological differences between males and females may lead to differing responses to health conditions, treatments, and environmental factors. These need careful consideration as there is limited research on how sex moderates physiological responses to microgravity. However, insight can be gained from ground-based investigations on sex-dependent health outcomes. For one, reproductive organs and endocrine responses can contribute to divergent health outcomes between the sexes. Osteoporosis is affected by age of onset, impacting the rates of bone mineral density and quality and rate of decline, which is different between the sexes. Moreover, prevalence of osteoporosis-related complications is higher in females but does occur in the majority of aging adults with osteoporosis and low bone mass in the United States [328, 329]. Notably, the prevalence of hip osteopenia and osteoporosis is 18% and 2% for men compared with 56% and 16% for women [330]. Similarly, females have greater incidence of cardiovascular disease-related deaths compared to age-matched males [331]. While these diseases are part of aging and affect older individuals in the general population, space flight physiological changes are similar to accelerated aging [332]. Additionally, the younger populations also demonstrate sex-specific physiologic outcomes. Prolonged energy deficit, resulting from inadequate energy intake and/or excessive exercise, can lead to reproductive suppression and poor bone health in females (i.e., Female Athlete Triad [333]) and males (i.e., Male Athlete Triad [334, 335]). However, there is indication that males are more resilient when faced with energy deficit. For example, in males, a greater deficit is necessary to impact reproductive and bone health and males experience a rapid recovery of reproductive function when the deficit is corrected [334, 335]. Dietary intake during flight is about 80% of the World Health Organization estimated requirement and may contribute to the body mass that is lost rapidly during the first 30 days of flight (about 2.8–4.4% loss) [336]. Male astronauts may be better able to withstand the caloric deficit common during flight without significant physiological implications. It is clear from clinical examples that many aspects of musculoskeletal and

cardiovascular health are moderated by sex, which may lead to varying physiological responses to space flight, as displayed in Figure 47. Ultimately, limited research has focused on the female astronaut and limited investigations on the implications of space flight on human physiology from a sex-dependent lens, leaving important gaps in our understanding of how to protect and maintain female astronaut health and performance.

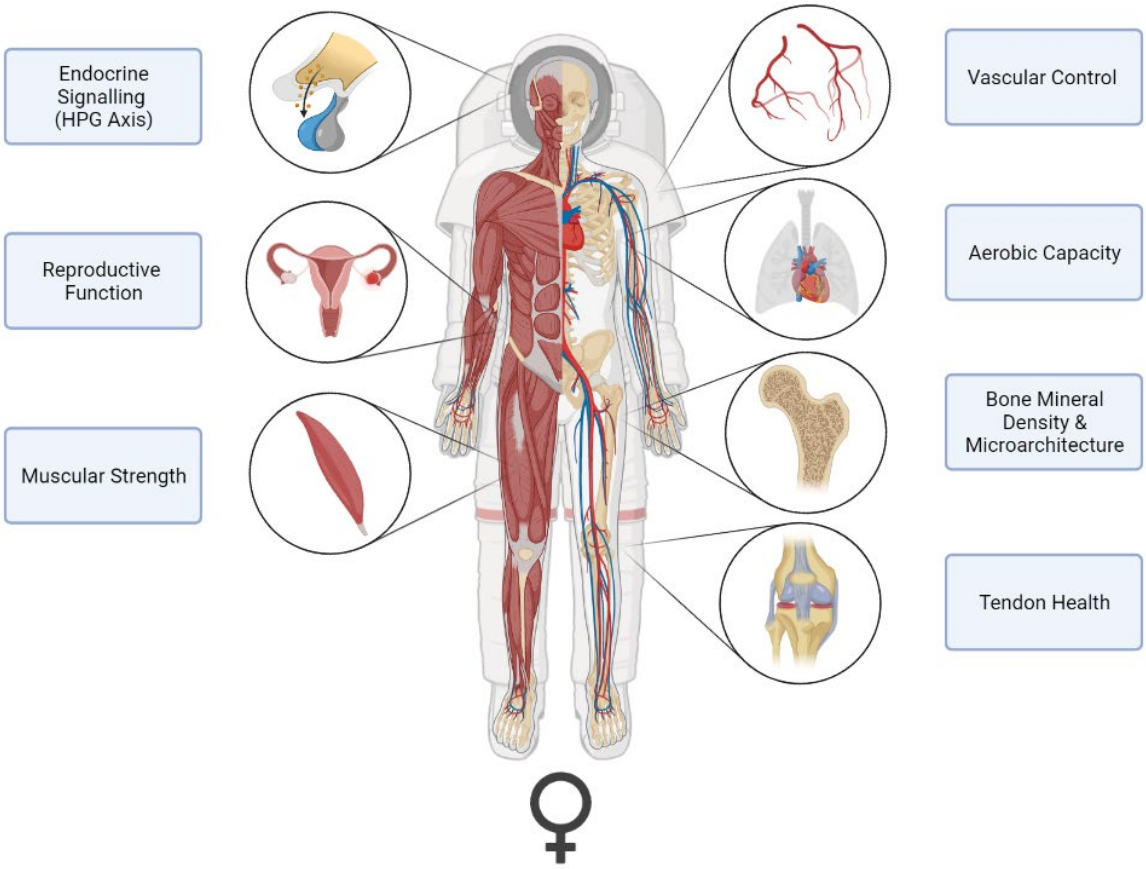

Figure 47. *Physiological systems and factors related to musculoskeletal health and aerobic capacity implicated with spaceflight.*

i) Hormones and metabolism

The Hypothalamic-Pituitary-Gonadal (HPG) axis is the primary signaling pathway responsible for controlling reproductive function in females and males. Within the brain, gonadotropin releasing hormone produced by the hypothalamus stimulates the anterior pituitary gland to release luteinizing hormone and follicle stimulating hormone. These hormones ultimately regulate gonadal steroid production, initiating the secretion of estrogen and progesterone from the ovaries in females, and testosterone from the testes in males. Females, in particular, experience cyclic fluctuations in ovarian hormone concentrations across the menstrual cycle, where a normal cycle last between 21–35 days, with two distinct phases (follicular and luteal) separated by the release of an egg during ovulation [337]. Importantly, gonadal steroid hormones have wide ranging functions and have been implicated in bone health [338], cardiovascular

function [331], and immune function [339] while having sex-dependent sensitivity to stress [340] and energetic status [333, 335].

Dependent on the stimulus, the HPG axis responds to exercise in a physiologic or pathologic manner [341]. At present, NASA astronauts participate in moderate-to-high intensity exercise 6 days/week in-flight, with an allotted daily exercise time of 2.5 hr (including time for set-up, stowage, and personal hygiene). These activity levels exceed the recommended physical activity guidelines set forth by the American College of Sports Medicine [342] and the U.S. Department of Health and Human Services [343]. Therefore, understanding how exercise (or exercise-related energy status) impacts the body is an important consideration for highly active astronauts. In men, the HPG axis hormonal response appears to be dependent on duration [344] and intensity of exercise [345]. For instance, acute bouts of aerobic and resistance exercise increases total and free testosterone [346, 347], while excessive training and/or inadequate energy intake can result in decreased testosterone concentrations in men [346, 348]. In women, while endurance training has no impact on testosterone concentrations [349], greater daily exercise has been associated with increased ovulation [350]. However, a chronic energy deficit in exercising women is a causal factor for reproductive hormone disruption (i.e., decrease luteinizing hormone pulsatility, estrogen, and progesterone concentrations [351]) and resultant menstrual cycle disturbances (i.e., luteal phase defects, anovulation, and amenorrhea [352]), which can be caused by low daily caloric intake inadequate to meet exercise energy expenditure needs. Understanding external factors that can impact HPG axis regulation is an important consideration for developing exercise and nutritional countermeasures to maintain health and performance in all astronauts.

ii) Hormones & Menstrual Characteristics

Microgravity alters the HPG axis endocrine response, although much of the evidence has been in male animal models and astronauts. In early studies of male rodents, concentrations of testosterone in plasma [353], serum [354], and testicular tissue [353] were reduced with short-duration (2 weeks) exposure to microgravity. In male astronauts, testosterone responses to space flight vary depending on length of mission. Short-duration space flight of 12–13 days have resulted in a 30% reduction in testosterone concentrations, while longer duration flights did not result in reduced concentrations until landing, where total testosterone, free testosterone, and bioavailable testosterone decreased by 40–50% [355]. The higher cortisol concentrations were related to reduced testosterone on landing day [355], indicating that stress may be contributing to landing day decrements.

Few studies have focused on the impact of space flight and simulated microgravity on female reproduction. In mice, hindlimb suspension and diet have been documented to impact time spent in estrus and plasma estradiol concentration [356]. Short-duration space flight (13 days) induced cessation of cycling while impacting ovarian physiology, including loss of corpus luteum and significantly reduced uterine estrogen receptor mRNA levels [357]. Conversely, during 37 days of microgravity aboard the ISS, mice continued or regained estrous cycle activity, albeit with decreased progesterone production during the extended duration [358]. To our knowledge, no study has reported alterations in menstrual cyclicity or reproductive physiology in response to space flight in female astronauts. However, simulated weightlessness through 6° tilt bed rest has been shown to have minimal impact on menstrual cyclicity, with one investigation demonstrating no effect in menstrual cycle length [359], while another reported luteal phase defects in female participants [360]. Notably, study length was not adequate (17 days) to confirm impacts on menstrual cyclicity [359]. Further research is necessary to determine whether space flight and

simulated microgravity impact menstrual cycle status, which may have downstream implications for estrogen-mediated physiological processes.

iii) Hormonal Contraception

Other considerations for female health include the use of hormonal contraception during spaceflight. Commonly, combined oral contraception of estrogen and progestin are utilized to suppress the menstrual cycle during missions [361, 362]. Benefits to hormonal contraceptive use during space flight include reduced menses and associated hygiene products, dysmenorrhea (i.e., painful menstruation), and total menstrual flow, with the latter potentially minimizing the temporary loss of red blood cell mass that is associated with spaceflight [362]. Additionally, hormonal contraception may be used as a prophylactic measure to help mitigate risk of gynecological pathology, such as abnormal uterine bleeding and endometrial disorders, during extended duration missions [363]. Because pregnancy is a contraindication to spaceflight, female astronauts may choose to suppress their menstrual cycle for extended periods, and it is estimated that the potential time frame of menstrual suppression by exogenous hormones could extend beyond a decade, to include candidate selection, astronaut training, awaiting mission selection, mission training, and space flight mission [361].

Notably, it is unclear how chronic use of hormonal contraception may impact other physiology, such as bone health. There are known route-dependent impacts of hormonal contraception on bone health, such as the reduced BMD associated with injectable depot medroxyprogesterone acetate [364], which is no longer prescribed to female astronauts due to a compounded risk of bone loss with space flight. With respect to oral contraceptives, the impact to bone health may be dependent on the type of estrogenic and progestogenic components, dosage of estrogen, and age of user [365-369]. Few studies have prospectively investigated the effects of long-duration oral contractive use on bone health. In an investigation examining the effects of hormonal contraceptive methods on BMD, women aged 18–33 y taking the desogestrel pill experienced a non-significant 2.6% reduction in lumbar spine BMD after 24 months, compared to age and race/ethnicity matched controls [370]. Another investigation in healthy women (19–22 y) found that oral contraceptive use over a 5-year treatment period did not change lumbar spine BMD, but it did prevent the almost 8% increase in BMD seen in control participants [371]. At present, it is difficult to distinguish whether hormonal contraceptive use during spaceflight in female astronauts is implicated in bone health, as no spaceflight investigation has explicitly examined bone health in this context. Medical recommendations for hormonal contraception use should be individualized based on health history and medical screening to balance the potential risks and benefits to the individual's health during spaceflight.

iv) Bone Health

Sex differences in the musculoskeletal system are well-documented, with males accruing greater bone, bone strength, and lean mass compared to females [372, 373] in adolescence. These differences are maintained throughout adulthood and are amplified with aging [374], particularly when females experience the drastic decline in estrogen production with the menopausal transition. While a sexual dimorphism exists in regulating musculoskeletal health on Earth, less is known to determine if there is a divergent response in bone health to space flight.

A majority of the research investigating bone loss with space flight has been reported in male astronauts, which can occur at rates of 0.8–1.5% per month [53, 375, 376] with the largest pre- to postflight decrements in BMD occurring at the spine/pelvis (−6%) and lower limbs (−5.4%) [53]. While few investigations utilizing 6° head-down tilt (HDT) bed rest to examine BMD

responses have been conducted in females, it is apparent that weight-bearing sites such as the hip and tibia are impacted with unloading. One 30-day HDT bed rest investigation was conducted in seven sets of female identical twins, in which the bed rest controls experienced BMD losses at hip and femoral shaft of 1.6% and 2%, respectively [377]. Longer duration (60 days) HDT in female participants (25–40 y) demonstrated the greatest losses of BMD at the distal tibia (−2.8%), total hip (−3.4–4%), and trochanter (−3.5%) [378, 379], which is estimated to be 20x the rate of bone loss seen in elderly populations [378]. Comparable decrements were reported in a 17-week HDT study, where female participants (n=5) had non-significant reductions in trochanter (−3.6%), total hip (−2.3%), and pelvis (−2.6%) [268]. In addition to BMD decrements, bone microarchitecture characteristics have also been shown to deteriorate with bed rest, as tibial trabecular density (2−.3%), outer trabecular density (−1.6%), and inner trabecular density (−3.2%) decreased compared to baseline, which persisted up to 1 year after bed rest [380].

To our knowledge, only one investigation has examined sex differences in the BMD response to space flight. The investigation by Smith et. al [381] demonstrated a comparable bone density response in male (n=33) versus female (n=9) astronauts who were evaluated before and after long-duration ISS missions, ranging from 49–215 d. Regardless of sex and exercise modality, total and regional BMD decreased up to 1.5% per month, and crewmembers with the greatest initial bone mineral content experienced the greater and faster loss of bone compared to those with lower BMC preflight [381]. While men had greater BMD compared to female astronauts through the study, as commonly reported in 1g literature, the sample size of the female cohort may not have been adequate to detect sex differences in response to space flight [381] as it has recently been suggested that sample sizes of 10–20 astronauts with >2 months of space flight is necessary to detect space-related changes in BMD [53]. The rate of bone loss (~1%/month at various sites) in presumably healthy women could have future implications to bone health later in life.

At present, the impact to bone structural qualities in female astronauts is unknown. In male astronauts, cortical and trabecular densities at the weight-bearing distal tibial decreased 1.5–2%, with 4% decrease in cortical thickness and 15% increase in cortical porosity after 4–6 months on ISS [375]. Additionally, preflight cortical area and thickness predicted in-flight cortical bone loss: the thinner cortical values at preflight, the greater the cortical bone loss. Importantly, some bone microarchitecture decrements due to space flight were not reversible in these male astronauts, as tibial cortical porosity and trabecular bone did not recover after 1 year of reambulation on Earth [375]. Similarly, recent research from Gabel et al. indicated that mission duration predicted loss and recovery of bone structural qualities, including trabecular BMD, cortical BMD, and trabecular separation at the distal tibia, such that longer duration missions resulted in greater loss and incomplete recovery [382]. While this study included female astronauts (n=3 of 17 astronauts included in analysis), sex differences were not investigated. The magnitude of sustained bone loss is comparable to a decade of normal age-related terrestrial bone loss. It remains unclear whether space flight has comparable long-term effects on BMD and bone microarchitecture in female astronauts; simulated microgravity appears to result in sustained decrements to weightbearing tibia trabecular bone [380]. Together, these findings are indicative of the prolonged impact space flight has on bone quality with potential to compromise bone strength; however, whether comparable decrements occur in female astronauts remains to be determined.

v) Tendon health

Muscle atrophy associated with the deconditioning due to space flight may have implications on tendon health. The Apollo 15 Commander experienced a muscle/ligament strain after heavy workload during lunar surface operations [383]. This may be of relevance to female

astronauts due to increased risk for ligament injury, particularly of the anterior cruciate ligament (ACL), with potential to impact the ability to complete tasks during lunar EVAs, egress from vehicle, and during emergency scenarios. In athletic populations, females have a 1.7x increased risk [384], or 3–8x relative risk [385], for ACL injury compared to males. Importantly, a high prevalence of non-contact ACL injury occurs in females [386] due to a combination of extrinsic and intrinsic factors. From a biomechanical standpoint, greater knee valgus is thought to contribute to increased risk [387]. Physiologically, the cyclic hormonal fluctuations throughout the menstrual cycle may also impact ACL tendon health, as several systematic reviews indicate greater ACL injury risk [388, 389] and ACL laxity [390] pre-ovulation, with the potential mechanism being increased estrogen concentrations reducing type I collagen synthesis [391, 392]. Whether oral contraceptive use influences ACL injury remains inconclusive [391, 392]. However, it is important to note that studies assessing menstrual cycle and oral contraceptive effects on ACL injuries are of low quality [393], warranting further investigation. At present, it is unclear whether exposure to microgravity (1/6 g) for lunar EVAs with varied terrain or return to Earth (1g), may lead to tendon injury or increased injury risk.

vi) Vascular control

The cardiovascular responses to stress are different between males and females [394] due to females having relatively smaller mass, heart and blood vessels, and lower blood volume [395]. In addition, because sex hormones (i.e., estrogen, progesterone, and testosterone) can alter the cardiovascular system [396], blood pressure and cardiovascular control are altered by estrogen on adrenergic receptors and may result in sex differences. For example, the increased estrogen concentrations in females can cause a blunted response to adrenergic stimulation, reducing the ability of the blood vessel to vasoconstrict in response to various stressors (i.e., exercise, movement from sitting to standing) [397]. The overall difference between males and females is that males have increased vascular resistance through an elevation of mean arterial pressure; whereas females have increased heart rate [398]. Additionally, young healthy females have an increased risk for orthostatic hypotension and presyncope symptoms compared to males. This has been reported to be associated with an attenuated cardiac function (i.e., decreased cardiac filling), rather than reduced responsiveness of vascular resistance during orthostatic tests [399].

Postflight orthostatic intolerance, or inability to stand without fainting for periods of time, has been well-documented [400, 401] and is more common in female astronauts [402]. Mechanisms for increased susceptibility in females with space flight are not fully understood, but distinct differences in sex steroid hormone profiles between males and females can influence vascular function and blood pressure regulation with implications for orthostatic intolerance. Steroid hormones affect vascular function, with estrogen playing a key role in arterial structure and endothelial function. Estrogen (i.e., 17b-estradiol) acts at the molecular level to impact vasodilation and endothelial remodeling, while also serving in an anti-inflammatory and antioxidant capacity to impact overall cardiovascular health [403-405]. Steroid hormone concentrations remain relatively stable in males from month-to-month; however, vascular function may fluctuate with changing estrogen concentrations throughout the menstrual cycle in females. A recent systematic review and meta-analysis indicated that endothelial function increased in the late follicular phase when estrogen concentrations are high but remains largely unchanged in the luteal phase when estrogen concentrations are low [405]. These cyclic fluctuations in ovarian hormones make it difficult to discern how estrogen modulates the baroreflex-mediated changes in heart rate and vasoconstriction. However, evidence suggests women with orthostatic intolerance have a reduced vasoconstrictor response to gravitational stress, which is further suppressed with estrogen treatment, and compensates with increased heart rate [406].

Following short-duration (5–16 day) missions, there is a high incidence of presyncope (28–100%) in female astronauts, compared to 7–20% in male astronauts [402, 407-409], which has been associated with low vascular resistance [402, 409]. Suggested mechanisms for this response include the indirect effect of estrogen on vasodilation in which women have less vasoconstrictive response to orthostatic stress and compensate with greater heart rate response [408]. Coupled with nearly three times greater plasma volume losses compared to male astronauts [409], low vascular resistance in response to orthostatic stress may limit the ability maintain blood pressure in female astronauts after space flight.

Females also demonstrate varied baroreflex sensitivity and blood pressure responses to lower body negative pressure (LBNP) and bed rest. Females have been reported to be less tolerant to LBNP, despite comparable cardiovascular and autonomic response to orthostatic stress among the sexes [410, 411]. However, menstrual phase (follicular versus luteal) appears to have no effect on response to LBNP [412]. In response to short-duration (<10 min) head-down tilt, female participants have lower head oxygenation compared to males [413]. Longer bed rest (7 days) resulted in divergent blood pressure responses to LBNP, with diastolic blood pressure increased in the male subjects but decreased in the females [414]. Parasympathetic modulation and baroreflex sensitivity have also been shown to decrease with 60-day bed rest, with women experiencing a larger decrease in baroreflex sensitivity by day 30 than men [415]. Together, there are clear sex-dependent differences in vascular control in response to space flight and simulated microgravity.

vii) Body mass and energy requirements

In general, the average woman has less body mass, with more body fat and less muscle compared to the average man [416], which translates to lower resting metabolic expenditures [417]. From a space flight perspective, female astronauts require less resources, have less exercise heat production, and less energy intake, than their larger male counterparts. However, inadequate energy intake is of concern for female astronauts which could have important implications for weight loss, physiological adaptations, EVA performance, and emergency egress.

Dietary intake during space flight is much less than predicted energetic requirements, possibly leading to reductions in body mass, hormone concentrations, lean tissue, and bone. In early missions, it has been reported that there is loss of more than 5% and as much as 10% of preflight body mass in male astronauts [418], largely due to inadequate caloric intake. More recently, crewmembers have been reported to lose 2–5% in the first month and maintain lower mass for the duration of the mission [336]. When energy intake is insufficient, such as with the male astronauts on Space Shuttle mission STS-55 who consumed only 60–85% of metabolic energy requirements, reproductive consequences can manifest, such reductions in circulating testosterone. Importantly, a 5–10% reduction in body mass in female astronauts could initiate a cascade of physiological adaptations to conserve the limited energy available [333], potentially leading to cessation of reproduction function and impaired bone health (i.e., Female Athlete Triad). Compounded with the known microgravity and radiation-induced decrements to bone density and quality, inadequate energy intake could contribute to further bone loss and increase injury risk. With long-duration exploration-class missions, where food resupply and menu fatigue could be problematic, sustaining adequate energy intake and maintain body mass is essential for the health of all astronauts.

The smaller body mass of female astronauts may have additional implications for EVA-related task performance. Spacewalks require the use of the protective pressurized suit which are not designed specific to body size, meaning that female astronauts are required to wear and carry

comparable suits to their male counterparts. Current EVA suit weight for ISS missions is approximately 319 lbs. [419], which imparts a significant strength and metabolic burden on smaller astronauts. For female astronauts, this could increase the susceptibility to fatigue, the time to completion for EVA-related tasks, and injury risk [407]. For upcoming lunar exploration, the Artemis Exploration Extravehicular Mobility Unit (xEMU) will be able to accommodate a wider variety of body sizes but is still estimated to be 405 lbs [420]. As 50% of the Artemis astronaut corps are female, the ability to use a better-fitting suit is critical for the successful completion of EVA objectives and reduced suited injury risk.

viii) Injury

Deconditioning due to space flight puts all astronauts at greater risk for injury, which could impact the ability to complete EVA-associated tasks or compromise an astronaut's ability to safely egress in contingency scenarios. Preliminary data for astronaut musculoskeletal injuries and diagnoses, including muscle sprains/strains, tendinitis/tendinopathy, fracture, and diagnosis of low bone mineral density, indicated that a majority of injuries occur postflight (92%), with far fewer occurring in-flight (6%) or at landing (1%). Most of the injuries reported were muscle strains/sprains (59%). Of the few injuries that occurred at landing, the muscle sprains/strains that occurred were localized to the back, neck, and shoulder, while the muscle sprains/strains that occurred in-flight were less localized. Postflight, muscle strains/sprains were the most common injury (55%), occurring mainly in the back, neck, shoulder, and leg. Tendon-related injuries were also prevalent postflight (21%), occurring mainly in the shoulder, elbow, wrist, and ankles.

Physiological differences in hormones, body structure, and stature may lead to varied rates of injury among males and females. On Earth, females are commonly more prone to injuries, particularly in the lower extremities, compared to males. Fewer total musculoskeletal injuries have been reported in female astronauts compared to males, most likely reflective of the fact that there have been historically fewer female astronauts. While in-flight injuries are less frequent overall, in-flight injuries made up 20% of all injuries reported in female astronauts, compared to 4% in males. Regarding postflight injuries, which made up 94% of male and 80% of female astronaut musculoskeletal injuries, muscle strains/sprains were comparably prominent in all astronauts. However, diagnoses of low BMD appeared higher in female astronauts (15%) compared to male astronauts (4%). Together, these data provide important information regarding the health of all astronauts and may be informative for the continued improvement of preventative exercise countermeasures to help minimize the risk of musculoskeletal injury (Table 10).

Table 10. *Musculoskeletal Injuries/Diagnoses*

	Male	Female
In-Flight	**10**	**8**
Muscle Sprain/Strain	*10*	*7*
Tendinitis/ Tendinopathy	*0*	*1*
Landing (R+0)	**5**	**0**
Muscle Sprain/Strain	*5*	*0*
Postflight	**229**	**31**
Muscle Sprain/Strain	*128*	*16*
Tendinitis/ Tendinopathy/ACL injury	*50*	*5*
Fracture	*41*	*4*
Osteopenia/Osteoporosis	*10*	*6*
Total Musculoskeletal Injuries/Diagnoses	**244**	**39**

Note: Demographic and injury data was collected as part of the Lifetime Surveillance of Astronaut Health (LSAH) program. Available data was queried from NASA astronauts who participated in Mercury missions through Expedition 66, as of August 2022.

ix) Menstrual cycle on performance variables

Currently, there is debate whether endogenous hormone fluctuations in menstrual cycle phases can impact aerobic and strength performance outcomes. Results from recent systematic reviews and meta-analyses indicates a trivial impact of menstrual cycle phase in regularly cycling women [421-423]. In particular, both aerobic and strength performance were minorly reduced in the early follicular phase (effect size= 0.06) [421]. Similarly, there appears to be minimal-to-no impact of oral contraceptive use on performance outcomes, compared to eumenorrheic women [424]. However, it should be noted that the overall quality of evidence of the studies included in these systematic reviews was low, with only 17% of studies being characterized as high quality [421, 424]. At present, more quality research is necessary to conclusively determine whether menstrual cycle status influences performance; however, recent investigations continue to confirm that neither menstrual cycle phase nor hormone contraception phase have little to no effect on performance [425]. Furthermore, it is currently unknown if an interaction exists between space flight and menstrual cycle on aerobic and strength performance outcomes; more research is needed to further our understanding on this topic, which may provide an opportunity to develop targeted countermeasures to maximize female astronaut health and performance during space flight.

x) Aerobic Capacity

While aerobic deconditioning is a known physiologic outcome of space flight [42, 47, 118], few studies have reported direct comparisons between male and females in response to microgravity. One such study examined data from male (n=30) and female (n=7) astronauts on response to aerobic exercise on ISS missions (mean: 163 day duration) [426]. During submaximal cycle ergometer testing, elevated heart rate was documented in both male (8–9%) and female astronauts (11–14%) early postflight (R+5), compared to preflight (L-270) values [426]. Additionally, the male astronauts had higher VO_2 values (1.04L/min @ 25% VO_2peak, 1.74L/min

@ 50% VO_2peak, 2.56L/min @ 75% VO_2peak) than females (0.83L/min @ 25% VO_2peak, 1.36L/min @ VO_2peak, 1.98L/min @ 75% VO_2peak), which was a result of higher workloads determined by preflight absolute VO_2peak values. In female astronauts, in-flight aerobic capacity index, which represented linear extrapolation of HR and VO_2 to estimate aerobic capacity, increased linearly throughout the ISS missions, and was 0.73 L/min lower compared to male astronauts. Due to low female sample size, results should be interpreted with caution.

Few studies have examined the effect of sex on the change in aerobic capacity after bed rest. One investigation has been conducted in females to determine the impact of 17-day HDT, in which submaximal VO_2 decreased 11.9% during exercise with a concurrent increase of 6.2% in submaximal heart rate after bedrest compared to control condition [359]. Mean plasma volume also decreased 12.6% after the bed rest condition [359]. While menstrual status was recorded throughout the intervention, with no changes in menstrual cycle length documented [359], the length of the study was not adequate to confirm the effect of simulated microgravity on menstrual cyclicity. Of the studies specifically examining sex differences in aerobic capacity with bed rest, male subjects had consistently higher pre-bed rest VO_2peak values than their female counterparts, and while the percent loss of VO_2peak was independent of sex in bed rest durations up to 30 days (Figure 48) (29, 38, 41, 77, 92, 97), the absolute decrease in VO_2peak generally is higher in males than in the female subjects [31, 187, 221, 222, 427]. Interestingly, recovery of maximal aerobic capacity after bed rest appears to be hampered in females, as females failed to recover the 13% loss even after 8–9 weeks of recovery [427].

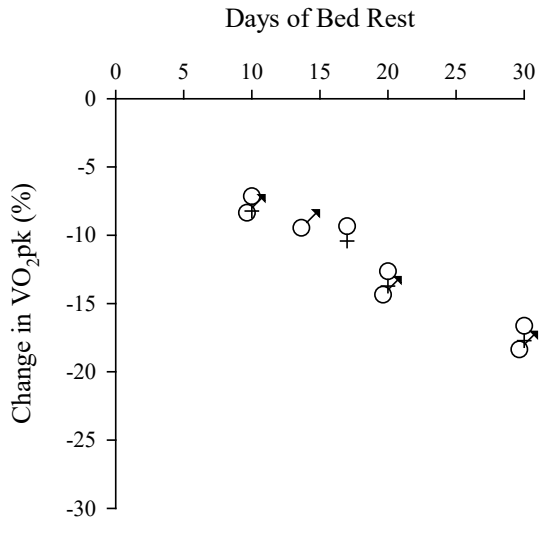

Figure 48. *The percent change in aerobic capacity (VO_2peak) after bed rest is not different between men and women [31].*

Preliminary data from ISS (Figure 49) show that VO_2peak is slightly reduced (but not significant) in females compared to males from pre- to postflight (Figure 48). However, aerobic power also showed that females have a greater reduction in maximal external work (females vs in males; $P<0.01$). It is unclear what is causing this disparity, but it may be due to differences in blood volume, vascular function,

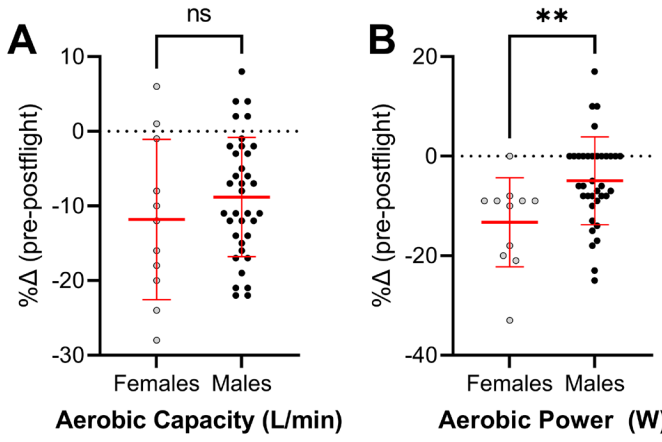

Figure 49. *Sex differences in aerobic capacity and power-preliminary data. VO_2peak (A) is similar between males and females; however, females have more loss in aerobic power compared to males from pre- to postflight. Sample size n=11 females, n= 36 males. ISS dataset pre- to postflight (L-3/1mo to R+3d), mission duration females 204±58 days and males 175±41 days, ns, not statistically significant at P<0.05. Mean ±SD, ** = P<0.01*

oxygen delivery, and skeletal muscle oxidative metabolism and requires further investigation. A larger sample size in the female cohort, controlling for age and flight duration, as well as further health and performance evaluations, are needed to confirm these preliminary findings.

xi) Muscle Strength and Endurance

Long-duration space flight and simulated microgravity result in muscle atrophy and strength decrements [428, 429], particularly in the muscles of the lower limbs critical to ambulation. In male astronauts, there is considerable variability in the muscle volume and strength decrements reported with space flight, with duration of mission and location of muscle contributing to the variable responses [57]. However, limited investigations consider sex differences when evaluating the extent of physical and functional decrements to muscle or the time course of alterations stemming from actual and simulated unloading. Functionally, only one investigation has examined strength changes in male and female astronauts, where it appears that females experience greater mean decrements to lower limb isokinetic strength following space flight [59]. However, explicit statistical analysis for sex differences were not performed and the 95% confidence intervals for all variables overlapped, suggesting that there was no significant sex effect on strength loss in response to space flight [59].

Muscle unloading consistently results in maladaptive changes to the neuromuscular system; unfortunately, few studies have examined the sex differences in muscular deconditioning with unloading. Short-duration interventions (7 day) demonstrated significant reductions in knee extensor isometric peak torque (22–28% reduction in females versus 13–16% in males) and isokinetic peak torque at various contractile velocities (7–19% reduction in females versus 0–11% in males), with total work (8–18% reduction) and average power (11–19% reduction) significantly impaired in female participants only following unloading [430, 431]. These results are consistent with longer duration intervention (14 day) in which females have greater reduction in knee extensor isokinetic strength compared to males (16.6% reduction in females versus 4.7% in males) after unloading [432]. Because sex-specific strength decrements have been reported, despite similar level of muscular atrophy [432], it is plausible that differing neural input may be driving these adaptations. In fact, reductions of 27–24% in electromyography activity have been documented in females, compared to 8% in males, following unloading [433]. These results indicate that the capacity to stimulate muscular contraction is impaired in females following unloading.

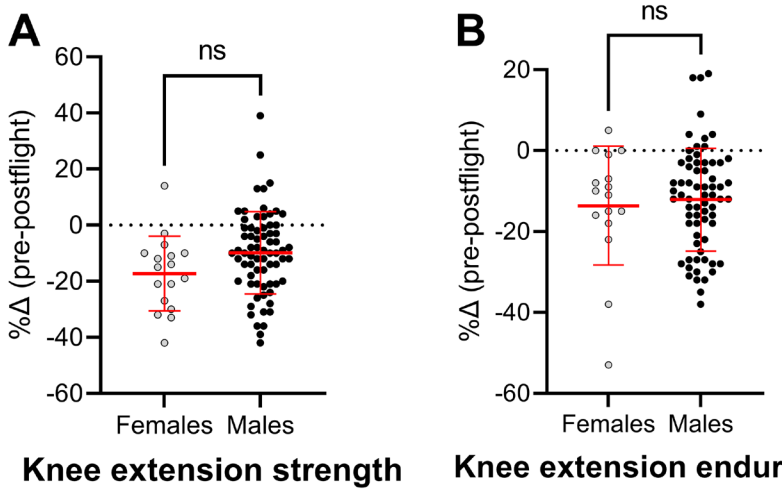

***Figure 50**. The reduced knee extension strength and endurance is similar between females and males pre- to postflight. ISS dataset pre- to postflight (L-3/1mo to R+5d). Note trending p-value for knee strength (P=0.06) likely due to small sample size (n=17 females, n= 70 males). Mission duration females 179±50 days and males 172±33 days, ns, not statistically significant at P<0.05. Mean ±SD.*

Importantly, the time course of neuromuscular impairment in women demonstrates that changes occur early in immobilization and may take longer to recover. Moderate to large decrements in isometric strength are noted within 48 hr (7.5%), with significant reductions at 1 week (10.6%) and 2 weeks (14.2%) in female participants following knee joint immobilization [433]. Similarly, isokinetic strength was reduced by 5.5%, 10.1%, and 9.7% at 48 hr, 1 week, and 2 weeks, respectively in these female participants [433]. Importantly, recovery from immobilization has been shown to be slower in women. Following 3 weeks of wrist immobilization, male participants fully recovered strength within 1 week, while female participants failed to improve and strength remained 30% lower than baseline values [434]. While the time course for strength decrements and recovery associated with space flight has yet to be determined in female astronauts, these investigations demonstrate the need for rapid implementation of exercise countermeasures to minimize deconditioning.

Preliminary findings from ISS data show a trend ($p=0.06$) in differences between males and females for changes in isokinetic knee extension strength after spaceflight but not for knee endurance (Figure 50). A larger sample in females, controlling for age and flight duration, is needed to confirm these preliminary findings.

o. Exercise Countermeasures

Table 11. *Historical progression of exercise countermeasures hardware and prescription of exercise*

Project	Mercury	Gemini	Apollo	Skylab	Shuttle	Shuttle-Mir	ISS
Years	**1959–1968**	**1961–1966**	**1961–1972**	**1965–1979**	**1972–2011**	**1995–1998**	**2000–present**
Exercise hardware	None	Bungee exerciser	Exer-genie Cycle ergometer	Cycle ergometer Teflon treadmill MKI/Mini gym/MKII	Cycle ergometer Passive treadmill EDO treadmill EDO rower	Cycle ergometer EDO treadmill	CEVIS TVIS iRED ARED T2
Exercise Prescription	No standardized in-flight exercise program. Used 30 s exercise session using a bungee cord with a 16-lb pull through	No standardized in-flight exercise program. Used 30 s exercise sessions with a bungee pull device with device delivering a force of 70 lbs.	No standardized in-flight exercise program. Exercised several times per day for periods of 15–30 min.	The Skylab 2 crew recommended that the personal in-flight exercise program be extended in both duration and type. To meet this recommendation, the exercise period for the Skylab 3 crew was expanded from one-half hr to 1 hr daily. On Skylab 4, the duration of crew exercise was further expanded to one and a half hrs daily and a unique treadmill device was used by the crew.	Regular aerobic exercise was defined as three or more sessions per week, each session lasting at least 20 min and at an intensity that elicited a HR of > 70% of their age-predicted maximum HR.	Regular aerobic exercise was defined as three or more sessions per week, each session lasting at least 20 min, and at an intensity that elicited a HR of > 70% of their age-predicted maximum HR.	Astronauts perform cycle ergometry, treadmill running, and resistance exercise during flight as prescribed by Astronaut Strength, Conditioning, and Rehabilitation specialists. Exercise prescriptions 6 days/wk of upper and lower body resistance exercise and 6 days/wk of moderate- to high-intensity aerobic exercise.

Note: Progression of exercise hardware and exercise prescription. CEVIS, Cycle Ergometer with Vibration Isolation and Stabilization; TVIS, Treadmill with Vibration Isolation and Stabilization; iRED, Interim Resistive Exercise Device; ARED, Advanced Resistive Exercise Device; T2, Treadmill 2.

Exercise is the only known countermeasure for protection of space flight deconditioning of crew health and performance. Table 11 shows the history and progression of hardware and exercise prescriptions from Mercury to current ISS missions. The optimal countermeasure prescription for the prevention of space flight-induced deconditioning should ideally include components to stimulate or maintain each organ system's condition similar to that maintained in a normal gravity environment, and it should require a minimal amount of crewmember time [435]. The total time currently allowed for resistive and aerobic exercise on ISS, including set-up and stowage of the exercise hardware and personal hygiene, is 2.5 hrs per day. Countermeasures that require too much time or are too intensive may reduce compliance in some crewmembers and may be difficult for schedulers to accommodate among various mission critical tasks. It is paramount that the countermeasures employed to protect crew health be of sufficient efficacy to promote and maintain high levels of function, such as aerobic and anaerobic fitness, in both male

and female astronauts. Care must be taken, however, when attempting to implement countermeasures that were successful in bed rest to the space flight environment due to logistical constraints of the space flight environment. In addition to crew time, exercise hardware mass, volume, and stowage should be considered as well as the impacts of countermeasure performance on the environmental control systems.

Although the preservation of VO_2peak and exercise performance after short-duration bed rest studies primarily may be achieved through protecting against blood volume losses and changes in SV, the maintenance of VO_2peak during longer bed rest exposures also likely requires the maintenance of aerobic pathway enzymes, muscle strength and endurance, neuromuscular coordination, muscle capillary density, and cardiac mass and function [178, 179, 208].

i) Exercise Prescription Aerobic

Exercise is a natural modality to consider when developing countermeasures to the decrease in VO_2peak during and after spaceflight and/or bed rest. Longer duration bed rest studies (>30 days) that employ an exercise countermeasure show mixed results regarding the ability to protect VO_2peak. In general, it appears that moderate to high intensity daily exercise is required to provide some level of protection of VO_2peak. While there are not ISS flight studies that have specifically looked at the effect of in-flight exercise frequency, time, or intensity on preservation of VO_2peak, Moore et al. showed that VO_2peak was better preserved in the astronauts who performed in-flight exercise at higher intensities compared to those who engaged in lower intensity exercise [47].

Data from the bed rest studies have shown that moderate intensities of aerobic exercise are not consistently effective to prevent the loss of VO_2peak. For example, Stremel et al. [169] were unable to prevent the decrease in VO_2peak and plasma volume during 2 weeks of bed rest when subjects performed two daily 30-min bouts of supine cycle ergometry at an intensity of 68% of pre-bed rest VO_2peak. However, Shibasaki et al. [251] maintained VO_2peak and plasma volume in during 14 days of bed rest with 90 min of daily exercise at 75% pre-bed rest HR. Short, intense bouts of exercise in ambulatory subjects are considered to be more effective than longer, less strenuous exercise in promoting changes in aerobic fitness in ambulatory subjects [436]; therefore, they are perhaps more likely to provide protection during bed rest. Greenleaf et al. [437] used a near maximal (up to 90% of pre-bed rest VO_2peak) interval exercise protocol (two 30-min bouts), 5 days per week during 30-days of bed rest to prevent the loss of both VO_2peak and plasma volume (−1%, NS). Control subjects in this study experienced an average decrease in VO_2peak of 18% [437]. The success of this exercise protocol in bed rest prompted NASA Astronaut Strength, Conditioning, and Rehabilitation Specialists to include this protocol in their exercise prescriptions for astronauts onboard the ISS. Similar exercise countermeasure protocols have been used successfully in bed rest studies by other investigators [2, 38, 187, 228].

In an attempt to develop a more time efficient exercise countermeasure protocol, Convertino et al. [94] had subjects perform a maximal bout of supine cycle ergometry as a simulation of exercise in microgravity at the end of a 10-day bed rest. Although VO_2peak measured during this supine ergometry test was significantly reduced from pre-bed rest (−5.6%), when subjects performed an upright treadmill test 3 hrs later, they exhibited no change in treadmill VO_2peak compared to the pre-bed rest measurement [94]. Later, it was shown that a single bout of intense exercise 24 hrs before resumption of normal ambulatory activities normalizes plasma volume (control: −16%, exercise: −4%, NS) [438] and protects LBNP tolerance [193] but does not prevent a decrease in VO_2peak [185]. It has been postulated that factors other than the exercise countermeasure, including readaptation to the upright posture, likely influenced the preservation

of treadmill [213] VO_2peak in the Convertino et al. study. Decreased muscle strength and endurance associated with bed rest deconditioning also likely affect maximal exercise performance, particularly during cycle ergometry testing when knee extensor muscles are greatly involved. For example, decreased local muscle fatigability in the calf muscles following 16 days of bed rest was correlated with a decrease in VO_2peak among control subjects [185]. However, few studies have directly assessed the use of a resistive exercise countermeasure to protect VO2peak. Stremel et al. [169] reported that subjects who performed two 30-min sessions of static leg extension exercise (21% MVC for 1 min followed by 1 min of rest) during a 14-day bed rest study experience a significant decrease in VO_2peak (−4.8%), but the loss appeared to be attenuated compared to both control subjects (−12.3%) and those subjects who had performed a moderate intensity aerobic exercise countermeasure (−9.2%) [169]. Similarly, when subjects in a 30-day bed rest study performed two 30-min bouts of maximal isokinetic exercise (10 s of work, 50 s of rest,15 min per leg) supine VO_2peak was not preserved (-9.1%), but the loss was half that experienced by the control subjects (−18.2%) [213]. This partial preservation of VO_2peak using resistive exercise alone suggests that muscle strength and endurance are significant contributors to aerobic exercise performance after bed rest. Additionally, other studies which have used aerobic exercise countermeasures to prevent the decreased VO_2peak following bed rest demonstrated a protection of muscle performance.

A recent in-flight and bedrest exercise prescription study (SPRINT study) evaluated VO_2peak and strength measures before and after 6-month space flight and 70-day bed rest [7, 52] completed either the high intensity/lower volume integrated Sprint resistance (3 days/wk) and aerobic (interval and continuous workouts, each 3 days/wk in alternating fashion) exercise program or the standard ISS countermeasure consisting of daily resistance and aerobic exercise during long-duration space flight. Post-study evaluation of VO2peak (−6 to 10%) had comparable decreases in both groups. Muscle performance including leg press total work, isokinetic upper and lower leg strength, vertical jump power, and maximal jump height, as well as muscle size were similarly in protecting the decrease in performance using both exercise prescriptions (SPRINT and Standard ISS). The Sprint high intensity/lower volume training may be a time effective program of space flight exercise countermeasure prescriptions.

The integrated training program consisted of high intensity, lower volume exercise 6 days/wk (3 days of resistance and 6 days of aerobic) [7, 52]. This program entailed the completion of high intensity interval aerobic exercise (3 days/wk) and continuous aerobic exercise (3 days/wk) on alternating days. Specifically, each of the three interval workouts was completed once per week (8 × 30 s intervals; 6 × 2 min intervals; and 4 × 4 min intervals); continuous aerobic exercise consisted of 30-min bouts. Resistance training followed an undulating periodized model and was performed on the same day as the continuous aerobic exercise. Most days, and when possible, continuous aerobic exercise was to be performed second, 4–6 h after the resistance exercise session to optimize adaptations. For the typical 6-month mission, resistance training was comprised of a single 24-week mesocycle. After an initial 2-week acclimatization period, load and repetitions were varied daily (high volume = 4 sets of 12 repetitions, moderate volume = 4 sets of 8 repetitions, low volume = 4 sets of 6 repetitions). See Tables 12 and 13 for aerobic and resistance SPRINT protocols.

ii) Exercise Prescription Resistance

Table 12. *Aerobic Exercise SPRINT Training*

Exercise	Work time	Repetitions	Intensity (%VO_2peak)	Intensity (RPE)	Rest
A	30 min	1	75	13 somewhat hard	NA
B	4 min	4	90	18 very hard	3 min
C	2 min	6	70, 80, 90, 100, 90, 80	13, 16, 18, 20, 18, 16	2 min
D	30 s	8	100	20 very, very hard	15 s

VO_2peak, peak aerobic capacity; RPE, rate of perceived exertion.

Exercise and nutrition are the primary interventions that have been employed in ground-based models to prevent unloading-induced changes in skeletal muscle. Exercise countermeasures are largely effective at preventing deleterious changes in skeletal muscle during unloading while nutritional interventions are only somewhat, if at all protective, particularly when employed in the absence of exercise. Because the scope of this risk includes the impact of changes in muscle mass, strength, and endurance, we will focus on resistance exercise countermeasures and will examine aerobic countermeasures only when they were employed in conjunction with resistance exercise.

Table 13. *12-Week SPRINT Schedule*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Week 1	A	B	A	C	A	D
2	A	B	A	C	A	D
3	A	B	A	C	A	D
4	A	B	A	C	A	D
5	A	B	A	C	A	D
6	A	B	A	C	A	D
7	A	B	A	C	A	D
8	A	B	A	C	A	D
9	A	B	A	C	A	D
10	A	B	A	C	A	D
11	A	B	A	C	A	D
12	A	B	A	C	A	D

Exercise A-D descriptions are located in Table 12.

In short-duration bed rest, resistance exercise (80–85% 1-RM) preserved muscle strength of the thigh and calf [17, 292]. Protection of muscle volume occurred through the maintenance of protein synthesis, which also likely influenced muscle strength [281]. Similarly, Akima et al. [267] were able to maintain isometric peak torque in subjects who performed daily maximal isometric contractions of the knee extensors during 20 days of bed rest. In long-duration bed rest (119 day), Shackelford et al. [268] preserved isokinetic muscle strength and observed substantial increases in isotonic muscle strength using an aggressive resistance exercise training protocol. During 90-day bed rest, a flywheel resistance exercise device capable of providing eccentric overload (i.e., loading that is greater during the descent phase of a lift than during the ascent) prevented the loss of muscle mass and strength in the thigh, with concurrent attenuated losses in the calf (−15% muscle volume versus −29% in controls), peak power, and displacement during a vertical jump [16, 439]. This differential response of the quadriceps (knee extensors) and gastrocnemius/soleus

(plantar flexors) to exercise countermeasures during unloading is a common finding. Using the same exercise device and resistance exercise protocol but with the addition of aerobic treadmill running (a vertical treadmill with lower body negative pressure, LBNP), Trappe et al. [28] replicated their previous results in the thigh (protection of both muscle mass and strength) and improved calf outcomes (−8% muscle volume and maintenance of strength) during 60-day bed rest in women compared to their resistance exercise-only countermeasure. The preservation of muscle mass and function in the thigh was facilitated by maintenance of MHC I and IIa single fiber size and function [440]. A recent bed rest study demonstrated that a combined, high intensity resistance and aerobic exercise program could protect aerobic capacity, leg press power, and quadriceps CSA over 14-day unloading [441].

Other resistance training modalities employed during bed rest include centrifugation (artificial gravity), vibration, and neuromuscular electrical stimulation. A 21-day bed rest study with 1 hr of centrifugation per day (resulting in producing 2.5 x g of longitudinal loading at the feet) attenuated decrements in both knee extensor and plantar flexor torque-velocity relationships and muscle fiber CSA in both the vastus lateralis and soleus [442]. Artificial gravity was unable to maintain total MHC mRNA content, or the slow to fast fiber type conversion, in the soleus [442]. Mechanistically, post-absorptive muscle protein synthesis was maintained in the vastus lateralis and soleus with centrifugation in contrast to controls that saw a 49% reduction in vastus lateralis muscle protein synthesis and a non-significant 22% decrease in the soleus [280].However, muscle protein breakdown was unchanged in both groups [280]. Resistance exercise prevented (thigh) or attenuated (calf) decreases in muscle CSA and isometric peak torque during 60-day bed rest, but the addition of vibration to the resistance exercise protocol did not improve muscle outcomes [25] and whole-body vibration alone was ineffective to prevent decreases in leg volume during even brief, 14-day bed rest [443]. However, in combination with resistance exercise, vibration during long-duration bed rest did show efficacy to prevent negative changes in bone [444]. Neuromuscular electrical stimulation was effective to prevent both a decrease in quadriceps CSA and increases in mRNA of several negative muscle regulators but was unable to protect muscle strength during a brief 5 d period of unilateral limb suspension [445].

In summary, resistance exercise is an effective countermeasure to ground-based, unloading-induced alterations in skeletal muscle although total protection of the calf muscles has proven somewhat elusive; high intensity (i.e., % concentric 1-RM) and eccentric loading equivalent to or greater than concentric loading (i.e., eccentric overload) appear to be key modifiers of exercise efficacy. Artificial gravity via centrifugation is a promising intervention for skeletal muscle during unloading, the efficacy of which will likely be improved with the addition of dynamic, resistance exercise movements during centrifugation. Whole-body vibration is an ineffective countermeasure for muscle during unloading and does not improve the efficacy of resistance exercise regimens although positive effects for bone have been reported when combined with resistance exercise.

Some general conclusions that can be drawn from the above ground-based human studies are as follows: first, terrestrial unloading models produce selective atrophy in the muscles of the lower limbs, especially the anti-gravity muscles; second, this response is greater in the extensor muscles than in the flexor muscles; third, muscle atrophy occurs quickly (within 7–14 days) in response to unloading; fourth, loss of muscle mass is paralleled by decrements in muscle strength and endurance, but strength losses are greater than volume losses; fifth, long-duration terrestrial unloading produces a slow-to-fast shift in absolute myofiber characteristics and alters the expression of MHC isoforms in human muscle so that an increase in MHC hybrid myofibers is observed, resulting in a faster phenotype; sixth, high intensity resistance exercise (ideally coupled with aerobic exercise) is highly (quadriceps) to moderately (calf) protective of muscle mass and strength; seventh, other countermeasures have demonstrated efficacies ranging from promising (centrifugation), to partial (nutrition), to poor (vibration). Examples of SPRINT resistance training protocol are shown below (Tables 14 and 15).

Table 14. *Resistance Exercise SPRINT Training*

Work	Repetitions	Sets	Intensity (RPE)	Rest between sets
Low	12	4	4	2 min
Moderate	8	4	5	3 min
High	6	4	6	4 min

RPE, rate of perceived exertion.

Table 15. *12-week Resistance Training SPRINT Schedule*

	Day 1	Day 3	Day 5
Exercises	**Squat, Heel Raise, Romanian Deadlift**	**Sumo Squat, Heel Raise, Deadlift**	**Single-leg Squat, Heel Raise, Romanian Deadlift, Sumo Deadlift**
Week 1	Low	Low	Low
2	Low	Low	Low
3	Moderate	Low	High
4	High	Moderate	Low
5	Low	High	Moderate
6	Moderate	Low	High
7	High	Moderate	Low
8	Low	High	Moderate
9	Moderate	Low	High
10	High	Moderate	Low
11	Low	High	Moderate
12	Moderate	Low	High

Low load = 4 sets of 12 repetitions; Moderate load = 4 sets of 8 repetitions; High load = 4 sets of 6 repetitions. Subjects should perform resistance exercise 3 days/week. On a typical 6-month mission, crewmembers completed this 12-week cycle twice, although Weeks 1 and 2 (familiarization) were not repeated.

iii) Other Exercise Hardware Countermeasures

Artificial Gravity. The concept that gravitational or gravitational-like stress alone will provide some protection against the decrease in VO_2peak associated with bed rest is not new. In the 1960s, several reports were published which suggested that the amount of deconditioning associated with chair rest was less than that observed following strict bed rest [446-448] . Later work demonstrated that exposure to a real or simulated orthostatic stress alone may attenuate the loss of upright VO_2peak during short-duration, but perhaps not a longer duration, bed rest studies. Four hrs of quiet standing or 3 hrs of peripheral fluids shifts induced by a reverse pressure gradient garment were partially effective in protecting exercise capacity during 4 and 15 days of bed rest, respectively [153, 435]. In contrast, subjects who were exposed to two 30-min sessions of centrifugation (+2Gz) daily during 4 days of bed rest or daily multiple bouts of LBNP (−35 mmHg) during one month of bed rest experienced a similar loss of upright VO_2peak as control subjects [449, 450]. These findings suggest that long-duration or more frequent exposures to orthostatic stress alone are necessary to protect against decreased post-bed rest exercise capacity.

Recently, NASA completed a 21-day bed rest study in which 15 male subjects were assigned to serve as controls or to receive an artificial gravity countermeasure generated by a short radius human-rated centrifuge (Dr. Alan Moore, unpublished results). Countermeasure subjects were exposed to 1 hr of artificial gravity per day, with a load equivalent to +2.5 G_z at the feet. The subjects performed upright cycle ergometer tests to measure VO_2peak before bed rest and on the first day of recovery. VO_2peak was reduced by 10% in the control group, but it was not significantly changed in the subjects who received the artificial gravity countermeasure (−6%, NS; Dr. Alan Moore, personal communication). Following bed rest, plasma volume was reduced (−9%) in both control and countermeasure subjects, and there were no differences between the groups [451]. However, the knee and ankle extensor muscle strength of the countermeasure subjects was superior to that of the control subjects, perhaps because the countermeasure subjects performed short range of motion knee bends and heel raises during the centrifugation to protect against presyncope (Dr. Vince Caiozzo, personal communication), which may have aided in the performance of cycle test after bed rest.

Combined Protocols. Protection against the loss of VO_2peak after bed rest is probably most effective when the simulated or real upright posture is coupled with exercise. The combination of orthostatic stress and even mild exercise reduces the countermeasure time requirement in bed rest by one half to produce a similar benefit [435]. The addition of a gravity-like stress during exercise training may be necessary to maintain upright exercise responses after space flight and bed rest [198]. Supine exercise may maintain plasma volume, but a gravitational component, real or simulated, may be required to maintain venous return and SV during post-bed rest exercise [437].

Centrifugation to simulate an orthostatic stress during cycle exercise has been successfully employed to maintain upright VO_2peak (Figure 51) [38]. Subjects who performed two 20-min sessions of combined exercise and centrifugation on alternating days of 20 days of bed rest maintained upright VO_2peak (−9±7%, NS), while those who did not perform the countermeasure experienced a significant loss (−27±7%). Countermeasure subjects exercised first for 20 min with a 0.8-1.4 Gz load at the heart while pedaling the cycle ergometer with a constant exercise intensity of 60 W. A 10-min rest period without exercise or centrifugation was then permitted before subjects began the second exercise session. Subjects experienced 0.3 g at heart level during this session and performed an interval exercise protocol similar to one which had been previously used to preserve upright exercise capacity during 14-days of bed rest [2]. In addition to protecting VO_2peak, cardiopulmonary responses to submaximal exercise, including HR and SV, were maintained in subjects performing exercise during centrifugation.

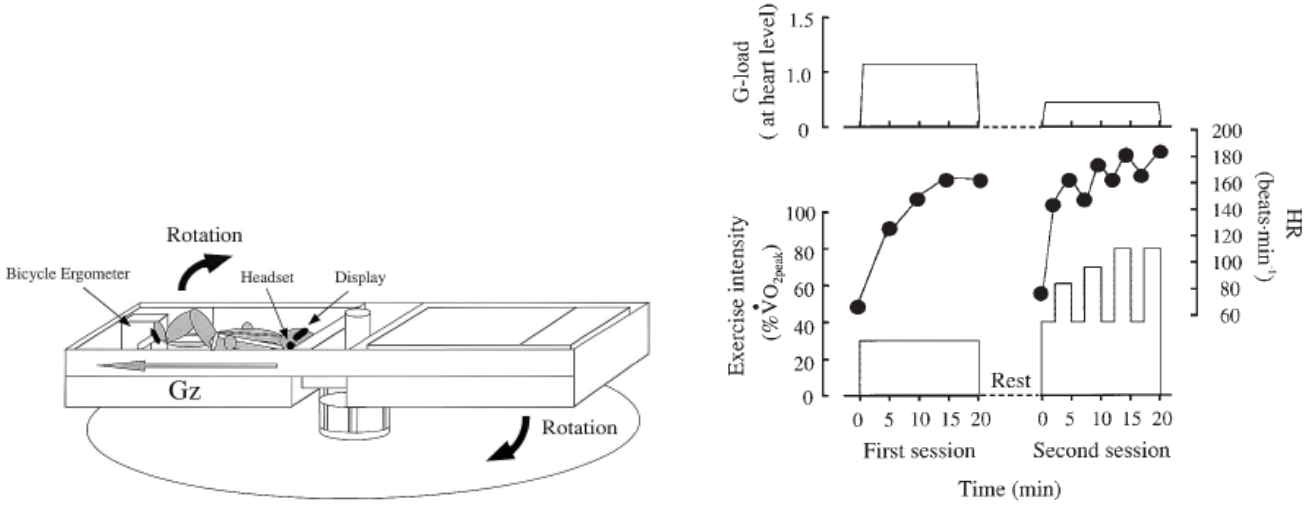

Figure 51. *Centrifuge with cycle ergometer and the countermeasure protocol utilized by Katayama et al. to preserve upright VO_2peak [38].*

Technical and logistical barriers to continuous whole space craft rotation or intermittent short-radius centrifugation make near term utilization of this centrifugation difficult; consequently, using lower body negative pressure (LBNP) to simulate orthostatic stress during an exercise may be an attractive alternative. The use of LBNP and exercise in separate sessions during bed rest was examined during a 28-day bed rest. The countermeasure subjects participated in a protocol of light supine cycle and isokinetic exercise and LBNP (−40 mmHg for 15 min per day) in the latter half of a 28-day bed rest. Countermeasure subjects appeared to receive some protection against loss of VO_2peak (−6% vs. Control: −16%, p=0.06) [452]. Also, plasma volume was maintained in the countermeasure subjects but significantly reduced in the control group [453].

The exercise during LBNP (Figure 52) was by a team of investigators led by Dr. Alan Hargens and Dr. Suzanne Schneider. The concept was developed in response to reports that long-duration crewmembers aboard the Mir space station exercise on the treadmill using loads equivalent to 60-70% of preflight body mass [454], which likely contributed to the inability of exercise countermeasures to fully prevent reduced VO_2peak [455], bone loss [456], postflight orthostatic intolerance [457], and decreased muscle mass, strength, and endurance [458]. Over the past decade the investigator team has documented the safety and effectiveness of a combined LBNP and treadmill exercise countermeasure. This integrated countermeasure method combines high loads on the musculoskeletal system with upright, Earth-like distributions of transmural pressure across blood vessels [10]. Subjects participating in these studies have comfortably run on the treadmill for up to 40 min daily at up to 1.2 body weight (~60 mm Hg) and experience dynamic loading with inertial forces on the musculoskeletal and cardiovascular systems similar to those present during upright exercise on Earth [459, 460]. In fact, metabolic and biomechanical responses of treadmill exercise within LBNP during simulated microgravity are comparable to metabolic and biomechanical responses of upright treadmill exercise on Earth [46]. The LBNP and exercise countermeasure system has prevented reductions in VO_2peak,

altered submaximal exercise responses, and decreased sprint performance during 5, 15, 30, and 60 days of bed rest [1-4].

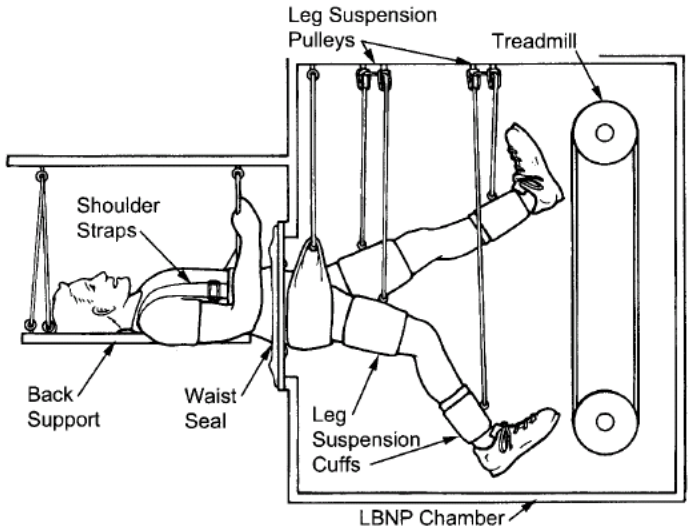

Figure 52. *Illustration depicts the lower body negative pressure.*

This (LBNP) exercise device used for supine treadmill exercise during bed rest durations of 5, 15, 30, and 60 days [1-4] . The device consists of a vacuum control system connected to a LBNP chamber enclosing a vertically oriented treadmill. A suspension system allowed subjects to perform treadmill exercise while supine by supporting their back and legs against the downward force of gravity. A broad, flexible neoprene waist seal spans the area between the subject and the edge of the elliptical opening. The waist seal area was to equal twice the subject's waist cross-sectional area, such that the negative pressure necessary to produce one body weight equaled −50 to −60 mmHg.

The LBNP and exercise device was first tested in a 5-day bed rest study [228]. Countermeasure subjects performed an interval exercise protocol modeled after one which successfully prevented a decrease in supine VO_2peak [213] and protected plasma volume [437] during 30 days of bed rest. The LBNP and exercise subjects exercised daily for 30 min against LBNP which provided one body weight of loading (mean: −51 mmHg). After the exercise, both the upright and LBNP and exercise subjects stood (LBNP and exercise subjects experienced LBNP without exercise) for 5 min. The length of the bed rest was insufficient to observe a consistent change in upright VO_2peak in the control group, but the submaximal exercise HR, respiratory exchange ratio, and ventilation were elevated. These changes during submaximal exercise were not evident in the LBNP and exercise group. LBNP and exercise training also prevented a decrease in plasma volume, which was observed in the control group, and protected against a decrease in tolerance to 30 min of head-up tilt [461].

The LBNP and exercise countermeasure was tested again during 15-days of bed rest in seven subjects using a cross-over design [2]. The exercise protocol was modified by increasing the duration of the high work stages (3 vs. 2 min) and the total exercise time (40 vs. 30 min), but the target intensities were somewhat less than in the 5-day study (peak intensity 80% vs. 90% pre-bed rest VO_2peak). The post-exercise LBNP exposure was not utilized in this project, but the amount of loading provided by LBNP was increased during the study to subject tolerance (1.0-

1.2 body weight). In the no exercise (control) condition, subjects experienced a significant decrease in VO_2peak (-14%) but had no significant change in VO_2peak after bed rest when they performed the LBNP and exercise countermeasure 6 days per week (−5%; Figure 52). Muscle performance also appeared to have been protected by these countermeasures; the time required to sprint 27.4 meters and plantar flexor muscle strength were maintained in the countermeasure subjects, while sprint time increased and plantar flexor strength decreased in the control condition. Additionally, the countermeasure attenuated the post-bed rest decrease in orthostatic tolerance, as measured using a progressive LBNP protocol, compared to the losses experienced by the control subjects [209].

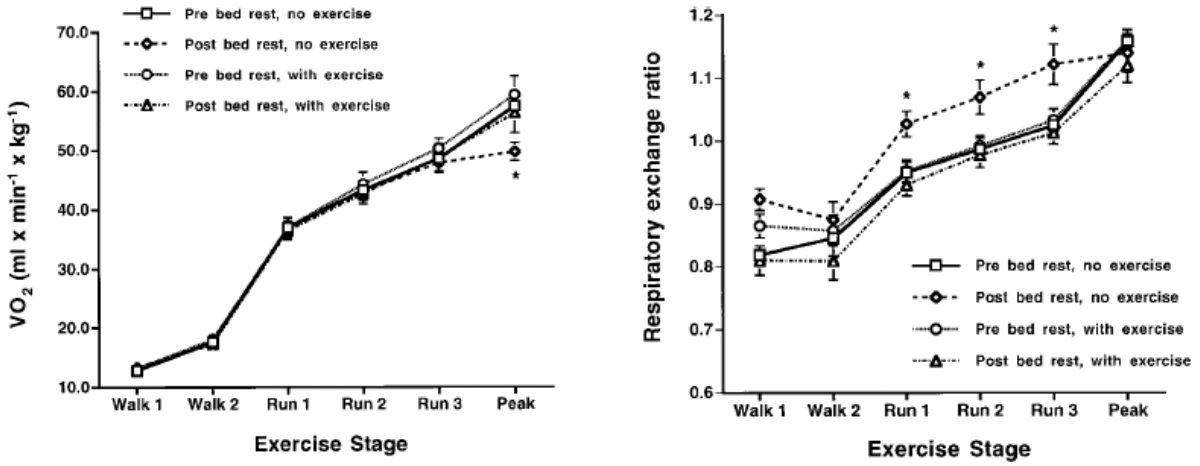

Figure 53. *Submaximal and maximal oxygen consumption (VO_2) and respiratory exchange ratio before and after 15 days of bed rest with and without an LBNP and exercise countermeasure [46].*

The LBNP and exercise countermeasure was later tested in male and female twins, one serving as the control with the sibling serving as the countermeasure subject, during 30 days of bed rest [4, 31]. The countermeasure protocol was the same as previously described in the 15-day bed rest study [2], and the post-exercise LBNP exposure utilized in the 5-day study was also implemented [228] (Figures 53 and 54). The investigative team hypothesized that the post-exercise orthostatic stress when the skin and muscle bed were near maximally dilated were helpful in preserving orthostatic tolerance [209, 210]. VO_2peak was decreased in the control subjects after bed rest (−18%) but not in the LBNP and exercise subjects. The time required to sprint 30.5 meters and knee, ankle, and trunk extensor muscle strength were also maintained in the countermeasure subjects but not in the controls [462, 463]. Performance of the LBNP and exercise countermeasure protocol also attenuated the decrease in orthostatic tolerance. During head-up-tilt at sub-tolerance levels of orthostatic stress, SV and HR during head-up tilt were maintained after 30 days of bed rest in the countermeasures subjects [210].

The LBNP and exercise countermeasure was tested during 60 days of bed rest (WISE-2005: Women's International Space Simulation for Exploration), in which countermeasure subjects also performed a resistive exercise protocol. Countermeasure subjects performed the LBNP and exercise protocol an average of 3 days per week and performed supine leg press and calf press on alternate days. The same exercise protocol applied in the 15- [2] and 30-day bed rest studies [4] was utilized during LBNP and exercise sessions, but the duration of the post-exercise LBNP stress was increased to 10 min. The resistive exercise protocol was fashioned after a countermeasure protocol which was successful in preserving muscle strength and volume in male subjects during 29 and 90 days of bed rest [16, 266]. During a treadmill walking test on the first

day of recovery, submaximal exercise responses were preserved in the countermeasure subjects but were elevated in the controls. When a maximal treadmill exercise test was conducted on the third day of recovery, VO_2peak in the countermeasure subjects was not different than pre-bed rest (−3%, NS), although VO_2peak was significantly decreased in the control subjects (−21%). Unlike previous work, sprint performance was not tested in this study, but ventilatory threshold was determined to be preserved in the countermeasure subjects and decreased in the controls. Knee extensor muscle strength and endurance [464] and ankle extensor strength [28] also was preserved with this countermeasure. Additionally, LBNP plus resistive exercise prevented cardiac atrophy in women during a 60-day bed rest. Left ventricular volume and long axis length was maintained in the countermeasure subjects during bed rest, and left ventricular mass, right ventricular mass, and mean wall thickness increased in these subjects [179].

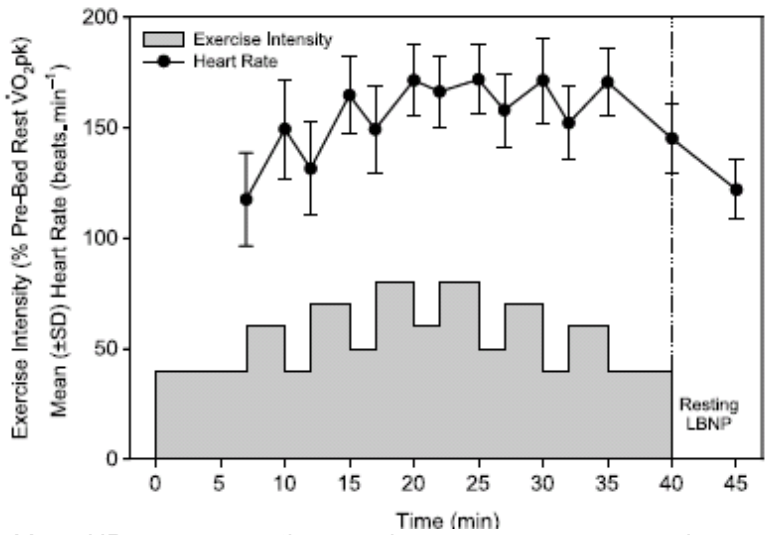

Figure 54. *Mean HR response to the exercise countermeasure sessions performed by the male countermeasure (exercise plus LBNP) subjects during 30 days of bed rest from [4]*

Unfortunately, presumably due to the large budgetary requirements of performing bed rest studies with multiple groups, none of the investigations which have used a countermeasure combining orthostatic stress and exercise have utilized either a group who were exposed to orthostatic stress alone or exercise alone. Consequently, it is impossible to determine the proportional contributions of the countermeasure components, exercise alone, orthostatic stress alone, or their combination, on post-bed rest exercise performance.

Adjunct countermeasures. With the increasing duration of exploration class missions (i.e., Artemis and Mars missions), additional countermeasures are necessary to protect astronaut musculoskeletal health and performance, as exercise countermeasures alone may be insufficient to protect against the functional decrements associated with long-duration space flight. In additional to nutritional strategies, pharmacological approaches for the protection of muscle and strength have been investigated. In particular, low dose testosterone treatment has been shown to be a safe and effective method for protecting against muscle atrophy in clinical populations and offers to be a promising adjunct countermeasure to exercise prescriptions during space flight. In order to determine the efficacy of concurrent testosterone and exercise treatment to prevent loss of skeletal muscle mass and strength during a long-duration space flight, 24 healthy male

participants (24-55 yr) completed a 70-day bed rest study where they were randomly assigned to a non-exercising + placebo control group, an exercise + placebo group, or an exercise + testosterone group [465]. Exercising participants followed the SPRINT exercise protocol 6 day/week [7], and the low dose testosterone treatment (100 mg/wk) was administered via intramuscular injection in 2-week intervals. While control participants lost 7.8% leg LBM, participants receiving with testosterone treatment improved 3.6% by study completion [465]. Similarly total LBM and trunk LBM were increased with testosterone and exercise treatment, while the controls showed decrements in these measures. Regarding knee extension and flexion strength, both exercise groups were similarly protected compared to controls. Additionally, exercise + testosterone treatment offered more robust protection for concentric and eccentric ankle plantar flexion strength (−3%) compared exercise alone (−11-14%), in relation to the decrements in strength reported in the control group (−24-30%) [465], which fall below the threshold for acceptable 20% reduction in strength with space flight outlined in the NASA standards. Together, these results suggest that cycled testosterone treatment is a safe and effective adjunct countermeasure to aerobic and resistance exercise in the maintenance of muscle mass and strength in males during simulated microgravity; however, more research is necessary to understand whether similar protection can be offered with varied exercise protocols and whether these findings can extend to a female population.

p. Other Organisms (animal, cells)

The goal of this section is to provide insight on animal research pertinent to the risk of impaired performance due to reduced muscle mass, strength, and endurance of the skeletal muscle system. This section will focus on two primary themes. Theme I will address the historical information that has been accumulated from space flight studies and ground-based analogues of skeletal muscle unloading, such as the hindlimb suspension (HS) model. Theme II will address recent studies, from 2008 to the present time, concerning the mechanisms impacting skeletal muscle atrophy along with exercise and molecular strategies designed to ameliorate muscle wasting. In the context of this presentation, the authors call attention to three key publications that provide important information impacting animal research relevant to the Human Research Program. The first involves the recent Decadal Study Report, “Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era”, published by the Space Studies Board of the National Academies in 2011.

In addition, the authors point out two recent review articles relevant to skeletal muscle homeostasis and muscle wasting by Baldwin et al. [466] and Brooks and Myburgh [467]**.** Although it is beyond the current theme of homeostasis in skeletal muscle, the authors encourage the readers to examine the exciting recent findings of Michael Delp and colleagues concerning mechanisms and functional consequences of vascular remodeling in skeletal muscle by Stabley et al. [468] and Sindler et al. [469].

i) *Historical Research Involving Animal Space Flight Studies and Ground-Based Analogs of Unloading*

This section summarizes the studies that have been conducted on animal subjects (such as rodents and non-human primates) that have been exposed either to space flight or (in the case of rodents) to the well accepted ground-based analog of HS to ascertain the effects of unloading states on the properties of muscle mass, strength, and endurance. The results presented herein overwhelmingly corroborate the body of evidence that has been reported on human subjects in

the preceding sections of this report. Importantly, using cellular and molecular analyses, greater insights have been obtained into the underlying mechanisms associated with these alterations in muscle structure and function. Since the majority of evidence concerning the effects of space flight on mammalian skeletal muscle has been derived from rodent studies, the information provided here is focused primarily on the rodent model. It is important to point out that the structure and function of rodent skeletal muscle are nearly identical to those of human skeletal muscle. For example, rodent muscle is composed of the same general fiber-type profile and is sensitive to the same environmental (mechanical, hormonal, metabolic) cues observed for human muscle. Thus, the information summarized below provides credence to the data base derived from human subjects. However, it is important to point out that one primary advantage of the rodent model is that adaptive changes occurring in both species unfold in a much shorter time frame in rodents than in humans (hours to days versus days to weeks), making it possible to predict long-term changes. Another important consideration in the context of animal research during space flight is that one can perform a straightforward experiment in which there is no requirement to provide a countermeasure intervention as there is for humans and can thereby avoid the introduction of a confounding variable in ascertaining the true effects of space flight on a wide range of physiological variables. Also, given the remarkable agreement in the quantitative and qualitative nature of the findings observed in the space flight studies versus those obtained from ground-based HS studies, we have chosen to combine and integrate significant portions of the data that have been gathered in the last 25 years. This rodent data base in space life sciences research includes 14 flight experiments with eight sponsored by the Russian Cosmos Program and six sponsored by NASA Space Life Sciences (SLS) and Space Transportation System (STS) missions [470-472]. These flight experiments are complemented by numerous ground-based research studies that focused collectively on the topics described below. Most importantly, all of the data reported in this summary are derived from animal cohorts in which the control animals were studied from a synchronous vivarium group of the same age, strain, and gender, and the analyses were performed at the same time as that of the experimental groups. The provided information is based entirely on peer-reviewed experiments as detailed in the bibliography provided.

Activity Patterns of Rodents during Space flight. While recorded observations during space flight are less extensive in rodents (due to fewer flight missions with opportunities for astronauts or payload specialists to observe them), the available data suggest that rodents rely less on the hindlimbs for executing most movement patterns (as is the case for humans). During space flight, their ankles appear to assume a plantar flexed position that may reduce the passive tension (force) imposed on the triceps surae group, of which the antigravity slow-twitch soleus muscle is a chief component [473]. A similar posture has been observed in the ground-based analog of HS. This posture is thought to affect the residual tension placed on this muscle group in the absence of a normal weight-bearing state, that is, the ankle plantar flexor muscle group becomes truly unloaded. While electromyographic studies on adult rodents have not been conducted during space flight, studies performed on rodents during chronic HS indicate that only a transient reduction occurs in electrical activity of the ankle plantar flexor muscles (soleus and medial gastrocnemius) [474]. This pattern of activity is consistent with the posture of the muscle and the maintenance of muscle mass during the 28-day time frame of the experiment. That is, the EMG activity was well-maintained, while the ongoing atrophy was maintained. These findings reinforce the notion that it is the mechanical activity rather than the electrical activity imposed on the muscle that is essential to maintaining physiological homeostasis of muscle mass.

Observations on Activity Patterns during Early Recovery from Space flight. When animals return from space flight of even short-duration (days), their basic activity patterns are altered. The center of gravity in rats is much lower than normal. They no longer support their body weight and

initiate movement off the balls of their feet, and the ankle joint assumes an exaggerated dorsiflexed position [473, 475]. Movement for most voluntary activities is much slower and more deliberate (the animals cover smaller distances per unit time), and the animals spend significantly less time in bipedal stances [473, 475]. Furthermore, investigator observation noted that the rodents use their tails for basic support to a greater degree. Thus, rodent motor skills and basic locomotor capability have less fidelity and capacity during posture maintenance and locomotion during the early stages of recovery; however, by 9 days after flight the activity properties return to those seen in normal conditions.

Effects of Space flight and Hindlimb Suspension on Muscle Mass, Protein Content and Gross Morphological Properties of Skeletal Muscle. Considerable information has accumulated covering a large number of space flight and HS experiments spanning a time frame of ~4 to 22 days for space flight and from 1 to 56 days for HS. These experiments have primarily focused on extensor muscles used extensively for postural support and locomotor activity. The review by Roy, Baldwin, and Edgerton provides one of the most comprehensive reviews on rodents in the space environment [476], and additional reviews on this topic have been published [477-483]. The collective observations clearly show that these types of muscles undergo significant reductions in muscle mass (i.e., muscle weight) [482-488], along with a concomitant loss in total protein and myofibrillar (the fraction that is composed of the contractile machinery of structural proteins) protein content of the targeted muscles [476, 479, 489, 490]. In some experiments, it has been reported that the myofibrillar fraction can be degraded to a greater extent than other muscle fractions [479]. The general pattern demonstrates that a rapid loss in muscle weight, net total, and myofibrillar protein content (concentration (mg/g X muscle weight) occurs during the first 7–10 days of unloading, followed by a more gradual loss in these constituents [470, 472]. The net result is that between 25 and 46% of the muscle mass can be lost in antigravity muscles of the lower extremity such as the soleus (a calf muscle) and vastus intermedius (a deep layered quadriceps muscle) [470, 472], which are composed mostly of the slow Type I myofibers containing the slow myosin heavy chain (MHC) protein. MHC is the most abundant protein expressed in striated muscle. This structural/regulatory protein serves as the motor protein that regulates, in synergy with its companion protein actin, the contraction process that derives the force, work, and power generation necessary for the muscle groups to bring about both movement and stabilizing types of activity (i.e., posture). It is also important to point out that fast-twitch synergistic muscles (expressing fast isoforms of MHC) are also targeted, but these muscles and their fibers are apparently not as sensitive to the unloading stimulus as the slower types of muscle are. Compared to both the slow and fast types of muscle, atrophy of the corresponding joint flexors, such as the tibialis anterior and extensor digitorum longus muscles in the leg, is markedly less [476]. Histochemical and immunohistochemical analyses at the single-fiber level clearly show that the atrophic process seen at the gross level is due to a reduction in the diameter of the affected myofibers of which the individual muscles are composed. These observations show that the slow type of fiber is more sensitive than the faster types of fiber, which is consistent with the gross muscle mass determinations [488, 491-493]. As a rule, regardless of the muscle, the larger fibers, whether fast or slow, are more sensitive to the unloading stimulus than their smaller counterparts [476]**.**

Muscle Fiber Phenotype Remodeling in Response to Space flight and Hindlimb Suspension. Accompanying the atrophy process noted above are the important observations that many (but not all) of the slow fibers in primarily antigravity-type muscles (e.g., soleus and vastus intermedius) are also induced to express fast myosin isoforms [472, 484, 485, 492, 494]. This transformation is largely manifested in the expression of hybrid fibers, in which both slow MHC and either fast type IIx or fast type IIa MHC become simultaneously co-expressed [484, 493]. These observations suggest that the slow MHC is targeted for degradation, evidenced by the net loss in slow MHC in

the atrophying muscle (fibers) [472, 479], while at the same time, according to pre-mRNA and mRNA analyses, up-regulation of the faster MHC genes by transcriptional and/or pretranslational processes occurs [492, 495-497]. More recent studies on this topic clearly suggest that the type IIx MHC, which is a faster isoform than the IIa type, is more abundantly expressed. From these observations it is apparent that the myofibrillar fraction, a key component of the muscle, is targeted for net degradation (as noted above) for two reasons: [1] degradation of this fraction allows smaller-diameter fibers to become manifest to meet the reduced requirements for force generation and [2] the unraveling of the myofibrillar system allows faster MHC isoforms to become incorporated into the contractile machinery to replace the slower ones so that the muscle is able to function more effectively under a reduced state of gravitational loading. Providing further insight is the observation that the unloading state of space flight and of HS also increases the expression of fast type II sarcoplasmic reticulum (SR) ATPase-driven calcium pumps (SERCA II) while repressing the slower type I SERCA calcium pump [498]. Since calcium cycling is used to regulate fiber activation and relaxation, the SR component of the muscle fiber controls the synchrony of contraction-relaxation processes. As calcium cycling and cross bridge cycling are the two major systems that account for the vast majority of the energy expended during muscle contraction to support movement, when this property of the muscle is switched to a faster system, the muscle can function more effectively in the unloaded environment. However, when the muscle encounters environments with a high gravitational stimulus, the faster properties are inherently less economical in opposing gravity, thus the muscle fibers become more fatigable when contracting against a load for long durations [485].

Metabolic Processes. In contrast to the contractile apparatus, studies on various rodent skeletal muscle metabolic enzymes have revealed a variety of responses with no clear-cut adaptive changes in oxidative enzyme expression [476, 477, 488, 493, 499]. These observations are consistent with the results of studies focusing on mitochondrial function after 9 days of space flight in which no reduction in the capacity of skeletal muscle mitochondria to metabolize pyruvate (a carbohydrate derivative) [480] was observed. These analyses were carried out under state 3 metabolic conditions, that , non-limiting amounts of substrate and cofactors, that simulate an energy turnover demand similar to that of high-intensity exercise [480]. However, when a fatty acid substrate was tested, a reduction in the capacity of different muscle types to oxidize the long-chain fatty acid, palmitate, was observed [477, 480]. This latter finding is in agreement with the observation that muscles exposed to space flight increase the level of stored lipid within their myofibers [493] and points towards the development of metabolic inflexibility [500]**.** Additionally, use of the metabolic pathway for glucose uptake is increased in muscles undergoing HS [493]. While the enzyme data are equivocal, it appears that in response to states of unloading, some shift in substrate preference may occur whereby carbohydrates are preferentially utilized based on utilization capability. If this is indeed the case, it could result in a greater tendency for muscle fatigue, should the carbohydrate stores become limited during prolonged bouts of EVA activity.

Functional Correlates to the Alterations in Muscle Mass and Contractile Phenotype in Response to Space flight. Stevens and associates [501] reported that in isolated single-fiber analyses, deficits in force generation capacity were found along with a reduced sensitivity to calcium stimulation. Similar observations occurred for both slow and fast ankle extensor fibers after 14 days of space flight. This study focused on the force-generating aspects of muscle fibers. It appears that only two additional studies have been conducted to examine the effects of space flight on rodent skeletal muscle functional properties using a more comprehensive set of analyses. One project was carried out for 6 days [484] while the other involved a 2-week flight (SLS-2) [485]. In both studies, the measurements focused on the force-velocity properties defining the limits of functional capacity of the muscle. These studies were conducted on the soleus skeletal muscle, in which slow-twitch myofibers predominate, because of the dynamic changes in fiber morphology

and phenotype that were observed in the other studies summarized above. Analyses on the animals were initiated within 6 hrs of return from space flight. The findings showed that the maximal strength of the muscle, as studied in situ using a computer-programmed ergometer system, was reduced by 24% after the 6-day flight and 37% after the 14-day flight [485]. These changes were consistent with the degree of atrophy observed at both the gross and single-myofiber level. Also, shifts occurred in the force-frequency response of the soleus in the flight animals, suggesting a switch to a faster contractile phenotype. Maximal shortening velocities were increased by 14% and 24% in the 6-day and 14-day space flight groups, respectively. These intrinsic increases in shortening speed were attributed, in part, to the de novo expression of the fast type IIx MHC in many of the slow muscle fibers. On the other hand, both work- and power-generating capacities of the flight-induced atrophied muscles were significantly decreased. Additionally, the resistance to fatigue was significantly decreased as well as was the ability to sustain work and power output in response to a paradigm involving repetitive contraction output [485, 502]. Similar findings have been observed using comparable analytical approaches involving the HS model [489, 491, 503]. Taken together, the findings clearly indicate that when skeletal muscles, especially those having a large proportion of slow myofibers, undergo both atrophy and remodeling of the contractile phenotype, the functional capacity of the muscle is reduced along with its ability to sustain work output. If a sufficient mass of muscle tissue across several key muscle groups were similarly affected, this would most likely impair the fitness of the individual when challenged with moderate-intensity exercise scenarios.

Are Atrophied Muscles Vulnerable to Injury? Riley and associates [475, 504] have provided an excellent synopsis of the structural integrity of mammalian muscle during the early stages after return from space flight. Their findings suggest that in atrophied slow types of skeletal muscle, there is no evidence of fiber damage when the muscles are taken from animals euthanized and processed during space flight. However, observations suggest that during the first 5-6 hrs after space flight (the earliest time point at which the animals can be accessed), edema occurs in the target anti-gravity muscles, such as the soleus and the adductor longus (AL) [475, 505]. This is thought to occur by increased blood flow to the muscles when they become initially reloaded in opposition to gravity. In addition, in certain regions of the AL, there is some indication of fiber damage based on histological analyses of the myofibril integrity and protein alignment in the sarcomere. While these observations were noted in ~2.5% of the fibers of the AL, they were not present in the soleus. Riley has proposed that the reason for the differential response between the two muscle groups is that weakened animals have altered their posture and gait so that eccentric stress is placed on the AL, resulting in some fiber damage. Edema and fiber damage were not noted in another cohort of animals studied 9 days after landing [505, 506]. However, in additional studies performed on both space flight and HS rodents [505, 506], in which 12 to 48 hrs were allowed to pass before the muscles were analyzed, observations indicated that the normal cage activity induced significant lesions in the muscles after sufficient reambulation was allowed. These included eccentric-like lesioned sarcomeres, myofibrillar disruptions, edema, and evidence of macrophage activation and monocyte infiltration (known markers of injury-repair processes in the muscle) within target myofibers [490]. The inference of these findings is that there is indeed a propensity for muscle injury secondary to the atrophic process that weakens the muscle, and—given the instability of the animal after space flight as described above—there is most likely a potential for injury if stressful stimuli are imposed on the muscle system before it can regain its proper structural and functional capability.

Cellular and Molecular Mechanisms of Muscle Atrophy in Response to Unloading Stimuli. As presented above, skeletal muscle atrophy involves an imbalance between the processes that control protein synthesis (also known as protein translation) and those that control protein breakdown. When the two processes are in synchrony, muscle mass is stable. However, if there

is an imbalance such that the protein synthetic pathway is decreased relative to that of the rate of degradation, muscle atrophy will occur. In the case of skeletal muscle atrophy in response to space flight or HS, a decrease in the capacity for synthesis as well as an increase in the processes that regulate degradation seem to occur, creating a rapid net degradation response to the unloading stimulus. On the basis of the available information, such a scenario is thought to involve the following chain of events. At the onset of unloading involving a wide range of models including space flight, a decrease in transcriptional and/or pre-translational activity occurs in skeletal muscle that affects the type I and IIa MHC genes as well as the actin gene [466, 472, 485, 495, 507]. This results in a reduced level of both pre-mRNA and mRNA pools (the latter being a substrate for protein translation) for these three proteins. Together, MHC and actin provide the bulk of the myofibril fraction that accounts for most of the protein in the muscle cell. Concomitantly, a decrease occurs in the activity of key protein kinase enzyme systems (constituting the PI3 kinase/akt/mTOR pathway), regulating the protein synthetic apparatus controlling protein translation [466, 508, 509]. This alteration, in combination with a smaller amount of mRNA substrate, collectively contributes to a reduction in the net capacity for protein synthesis. Occurring simultaneously with this process is the up-regulation of a set of genes that encode proteins that play a regulatory role in augmenting protein degradation. These include the myostatin gene [508, 509], the atrogin 1 gene [508, 509], and a gene called muscle ring finger protein, referred to as MURF [509]. Myostatin is an antigrowth transcription factor thought to negatively modulate the genes that promote growth. Atrogin and MURF are E3 ligases responsible for ubiquinating target proteins to mark them for degradation in a system designated as the proteasome. Interestingly, this MURF protein has been reported to be a key regulator for specifically targeting breakdown of the type I and type IIa MHC proteins [510]. As a result of the reduction in net capacity for protein synthesis and the augmentation of protein degradation, a net loss of muscle protein in the muscle fiber occurs along with a change in the relative proportion of the MHC protein content, since available findings show that the faster MHC genes are up-regulated during muscle atrophy [476, 478, 495]. Hence, this results in a smaller, faster muscle phenotype, which is apparently more suitable for muscle performance in states of unloading. The chain of events described above must be blunted or reversed if the muscle is to perform optimally when faced with an increased gravitational stimulus in returning to Earth or transitioning from low gravity (microgravity) to higher gravitational environments such as landing on the Moon or Mars. It is apparent that the best strategy to accomplish this task is via a vigorous countermeasure program to provide a high level of mechanical stress in preventing the imbalance in protein expression that occurs when the muscle is insufficiently loaded for significant periods without an intervening anabolic stimulus.

Effects of Space flight on Non-Human Primates. To our knowledge, the only other species besides the rat that has been involved in space flight studies on skeletal muscle is the rhesus monkey. Two monkeys were flown in space for 14 days on the Bion 11 satellite. They were compared to ground-based vivarium control animals as well as a chair-restricted group that involved immobilization of the upper arm and shoulder. The results from these studies provided the following insights. Individual fibers (slow and fast) of the monkey displayed functional properties more closely aligned to those of human fibers than to those of rodents, in that the fibers were larger but less powerful per unit cross-sectional area than rodent fibers [511, 512]. However, in pre- versus postflight analyses of single fibers, slow fibers in both the slow-twitch soleus and triceps muscles underwent greater atrophy and reductions in force and power production than fast-twitch fibers. Also, transformations in the myosin heavy chain profile indicated that there was a greater level of hybrid slow/fast fibers in the two different muscle groups [511, 512]. Immobilization of the triceps muscle group produced similar responses, but the magnitude of change was much less than that in the space flight animals [513]. Additional experiments were performed on these same animals. This involved locomotor activity before and after space flight

via muscle electromagnetic and tendon force recordings. These experiments demonstrated that postural and locomotor control was compromised by space flight as has been observed in humans [473, 512, 514, 515]. These alterations were chiefly manifested in modified load-related cues as reflected in the altered relative recruitment bias of flexor muscles versus extensors and fast versus slow motor unit pools. In an additional flight study (Cosmos Flight 2229) involving two rhesus monkeys, EMG recordings were obtained before, during, and after space flight [516]. These experiments were unique in that recordings obtained during space flight revealed a preferential shift in recruitment patterns favoring the fast medial gastrocnemius versus its synergistic slow soleus muscle; that is, the normal recruitment pattern was reversed [516]. This alteration was maintained well into the recovery stage after space flight, further suggesting a reorganization of the neuromotor system during and immediately after exposure to microgravity. Thus, it is apparent that skeletal muscle fibers of humans, monkeys, and rodents share similar patterns of myofiber alterations that, in the case of monkeys and humans, are also linked to altered motor performance in response to different states of unloading, reduced usage, and return to an Earth gravitational environment.

ii) *Mechanistic Studies of Relevance to the Human Research Program*

Effects of Space flight on Murine Skeletal Muscle Homeostasis. With the retirement of the Space Shuttle program, which enabled numerous studies concerning the role of gravity on skeletal muscle function and health in animal models, it is fortunate that Allen et al. [517] published an interesting article in 2009 impacting skeletal muscle gene expression in female mice (C57BL/6J) flown on the mid deck in animal enclosure modules lasting for 11 days and 19 hrs. on the Space Shuttle Endeavor (STS-108/UF-1). It has been previously shown that space flight results in numerous adaptations to skeletal muscle, including both muscle atrophy and shifts toward faster muscle fiber types (see theme section I). To identify changes in gene expression concerning these types of alterations, the authors used both microarray expression analysis and real-time polymerase chain reactions to quantify shifts in mRNA levels in the gastrocnemius muscle from the flight mice versus normal gravity controls. Space flight data also were compared with the ground-based unloading model of hindlimb suspension, along with another group of pure suspension and one of suspension followed by 3.5 hrs of re-loading to mimic the time between landing and euthanization of the space flight mice. Analysis of the microarray data revealed that 272 mRNAs were significantly altered by space flight, the majority of which displayed similar responses to HS; whereas, reloading tended to counteract these responses. Several mRNAs altered by space flight were associated with muscle growth, including the phosphatidylinositol 3-kinase regulatory subunit p85 alpha, insulin response substrate-1, the fork head box O1 transcription factor, and MAFbx/atrogin1. Moreover, myostatin mRNA tended to increase, whereas mRNA of the myostatin inhibitor FSTL3 tended to decrease, in response to space flight. In addition, mRNA levels of the slow oxidative fiber-associated transcriptional co-activator peroxisome proliferator-associated receptor (PPAR)-gamma coactivator-1α and the transcription factor PPAR-α were significantly decreased in the space flight gastrocnemius muscle (which is indicative of a decrease in slow fiber gene expression). Therefore, these interesting results became a catalyst for numerous ground-based research themes using the HS model as delineated below.

The Rapid Kinetics of Muscle Wasting in Response to Ground-Based Unloading Models. In previous reviews, as summarized in the theme I section of this report, it was clearly demonstrated that weight bearing muscle groups such as the ankle extensors in rodents are very sensitive to changes in loading state, especially during unloading conditions such as space flight and/or the model of HS. However, little is known of this process during the very early stages (hours) of unloading. Therefore, Giger et al. [495] characterized the dynamic changes in the unloaded rodent

soleus muscle in vivo following a short bout of HS and tested the hypothesis that transcriptional events are rapidly impacted by the atrophic stimulus. In fact, their observations demonstrated that after only one day of HS, primary transcript (e.g., pre-mRNA and mRNA) levels of skeletal alpha-actin and slow type I MHC genes were significantly reduced by more than 50% compared with ground control levels. The degree of decline for the mRNA expression of actin and type I MHC lagged behind that of the pre- mRNA after 1 day of HS, but by 2 and 7 days of HS, large decreases in mRNA for the two genes were observed. Although the faster MHC isoforms, IIx and IIb, began to be expressed in the soleus after 1 day of HS, a relatively significant shift in mRNA expression from the slow MHC isoform to the fast isoforms did not emerge until 7 days of HS.

Interestingly, one day of HS was sufficient to show significant decreases in mRNA levels of putative signaling factors such as serum response factor (SRF), suppressor of cytokine signaling-3 (SOCS-3), and striated muscle activator of Rho signaling (STARS); although transcription factors yin-yang-1 (YY1) and transcriptional enhancing factor-1 (TEF-1) were not as significantly affected. Interestingly, the protein levels of actin and type I MHC were significantly decreased after 2 days of HS, implicating that myofibril degradation is also being impacted early on during the atrophic stimulus. These alterations suggest the following: The synthesis side of the protein balance equation is rapidly down regulated; whereas the degradation process is most likely enhanced (see below) during the early stages of unloading. If these alterations that are occurring in these animal models are also occurring in humans (e.g., astronauts) it becomes apparent that it is critical that counter measures such as resistance training must be initiated early on during exposure to micro gravity environments.

Mechanisms of Slow to Fast MHC Gene Switching during Unloading: Role of Non-coding Antisense RNA. In previous sections of this report, we described that during unloading stimuli (HS model) there was a switching of MHC gene expression whereby the slow-type I and faster type IIa genes were repressed while the fast type IIx and IIb genes were expressed ne novo in the unloaded soleus muscle of rodents [470, 478, 484, 485]. Recall that the MHC gene family in striated muscle comprises at least eight members: two cardiac genes, alpha and beta, three adult fast MHCs (IIa, IIx, and IIb), two developmental MHCs (Embryonic and Neonatal), and one specialized type, i.e. the extraocular MHC (EO). Note that the slow cardiac beta MHC is the same as the type I MHC gene that is expressed in slow skeletal muscle fibers. As discussed in more detail elsewhere [466], these MHC genes are arranged into two clusters: 1) the cardiac MHCs on chromosome 15 in the rat and 2) the skeletal muscle MHC cluster on chromosome 10. This gene clustering orientation and tandem organization have been conserved through millions of years of mammalian evolution. The conserved configuration raises questions as to whether this particular MHC gene alignment is of functional significance in their patterns of regulation under different physiological states.

Recent evidence has implicated a non-coding RNA in the coordinated regulation of two positioned genes in tandem, indicating the importance of genomic organization of these MHC genes in their coordinated regulation. For example, in 2003, Haddad et al. [518] reported the novel discovery that in normal healthy rodent cardiac muscle, a naturally occurring antisense RNA transcript to the cardiac Beta (type 1) MHC gene is involved in cardiac gene regulation, such that the alpha MHC isoform normally is primarily expressed under normal physiological conditions [518]. Interestingly, cardiac alpha and beta MHC isoforms are the products of two distinct genes that are organized in tandem in a head to tail position on the same chromosome in the order of beta → alpha (e.g., the beta gene is upstream of the alpha), and they are separated by a ~4.5 kb intergenic DNA space [519]. In the normal state, a long non-coding antisense RNA is transcribed from the DNA that is opposite to the MHC genes creating a “beta antisense RNA”. This antisense-beta sequence was implicated in MHC isoform gene regulation/switching (alpha MHC repression

and beta expression enhanced) in the heart in response to both diabetes and hypothyroidism [520-522]. Under conditions of these conditions, the antisense Beta fragment was repressed allowing the beta MHC gene to dominate cardiac beta MHC compared to the normal heart. Given these findings, studies were subsequently performed on skeletal muscle to ascertain if the non-coding antisense RNA expression in slow and fast skeletal muscle contributes to the patterns of MHC gene expression in response to unloading stimuli.

In 2006, Pandorf et al. [496] published a paper which investigated type II MHC gene regulation in slow type I soleus muscle fibers undergoing a slow to fast MHC transformation in response to seven days of spinal isolation (SI), a model of inactivity that induces atrophy similar to HS [466]. Transcriptional products were examined of both the sense and antisense strands across the IIa-IIx-IIb MHC gene locus as depicted in [466]. Results showed that the mRNA and pre-mRNA of each MHC gene had a similar response to the SI stimulus, suggesting regulation of these three genes at the transcriptional level. In addition, detection of a previously unknown antisense strand transcription occurred that produced natural antisense transcripts (NATs). RT-PCR mapping of the RNA products revealed that the antisense activity resulted in the formation of three major products: aII, xII, and bII NATs, i.e., antisense products of the IIa, IIx, and IIb genes, respectively. Thus, the key observation of this experiment was that the SI-induced inactivity caused a marked inhibition of both the slow type I and type IIa genes along with upregulation of both the IIx and IIb genes. Therefore, the inactivity model of SI resulted in negatively impacts transcription of the type I MHC gene by inhibiting its promoter and induces anti sense aII NATS that primarily repress transcription of the IIa MHC gene thereby creating a switch from slow type I/IIa predominance to a fast IIx fiber of the normally slow soleus muscle. Importantly, this observation explains the existence of type I/IIx hybrid fibers reported previously by Caiozzo et al. [485], as presented in the earlier section of this review.

Mechanisms of Slow to Fast MHC Gene Switching During Unloading: Role of Epigenetic Modification of Histones at MHC Genes. Recent advances in chromatin biology have enhanced our understanding of gene regulation, especially the motor protein MHCs. It is now widely appreciated that gene regulation is dependent upon post-translational modifications to the histones which package genes in the nucleus of the cell. Active genes are known to be associated with acetylation of histones (H3ac) and trimethylation of lysine 4 in histone H3 (H3K4me3). Using chromatin immuno-precipitation (ChIP), Pandorf et al. [523] examined histone modifications at the MHC genes expressed in fast versus slow fiber-type skeletal muscle and in a model of muscle unloading (HS), which results in a shift to fast MHC gene expression in slow muscles. Both H3ac and H3Kme3 varied directly with the transcriptional activity of the MHC genes in fast fiber type plantaris and slow fiber-type soleus. During MHC with muscle unloading, histone H3 at the type I MHC becomes de-acetylated in correspondence with down-regulation of that gene, while upregulation of the fast type IIx and IIb MHCs occurs in conjunction with enhanced H3ac in those MHCs. Enrichment of H3K4me3 is also increased at the type IIx and IIb MHCs when these genes are induced with muscle unloading. Down regulation of IIa MHC, however, was not associated with corresponding loss of H3ac or H3K4me3. These observations demonstrate the feasibility of using the ChIP assay to understand the native chromatin environment in adult skeletal muscle, and also suggest that the transcriptional state of the types I, Ix, and IIb genes are sensitive to histone modifications both in different muscle fiber-types and in response to altered loading states.

Strategies for Ameliorating the Rapid Kinetics of Muscle Wasting. In 2009, Susan Kandarian's research group [497] using the HS model of unloading demonstrated that Nuclear Factor- Kappa B (NF-kB) signaling is necessary for the enhanced degradation occurring during the early stage of unloading-induced atrophy. Importantly, when this factor was inactivated, the

atrophy process was inhibited, suggesting that NF-kB plays a major role in the degradation cascade of the myofibril network during unloading.

In a follow-up study [524], the Kandarian group, using ChiP-gene sequencing technology, found that Bcl-3, an NF-kB transcriptional activator, is required for atrophy; this factor also binds to the promotors of a number of genes collectively involved in muscle wasting. By means of bioinformatics analysis of ChiP-sequencing data, they discovered that Bcl-3 directs transcription networks that includes many E3 ligases associated with the proteasomal protein degradation network, including that of the N-end rule pathway. These findings are important because they could enable a process to either slow down or prevent the acceleration of muscle wasting by inhibiting this critical pathway.

For example, atrogin-1 and MurF1 are muscle-specific ubiquitin ligases that play a pivotal in protein degradation by targeting myofibril protein for degradation during states of unloading. Interestingly, Maki et al. [525] tested the hypothesis that branched-chain amino acids (BCAAs) inhibit atrogein-1 and MuRF1 and have a protective effect on disuse muscle atrophy. To test this hypothesis, they used the HS Model. Their findings showed the following: 1) HS significantly reduced soleus muscle weight and the CSA of soleus muscle fibers. 2) Branched chain amino acid administration significantly reversed the HS-induced decreased in fiber cross sectional area. 3) While HS increased expression of atrogin1 and MuRF1, which are involved in muscle atrophy, branched-chain amino acid attenuated the increase in atrogen-1 and MuRF1 in the soleus muscles. Thus, further studies on this important finding are warranted.

In another interesting study, Derbre et al. [526] developed a strategy to determine the mechanism by which xanthine oxidase (XO) causes unloading-induced muscle atrophy in rats via HS along with its potential prevention by allopurinol, a well-known inhibitor of XO and a key therapeutic factor in preventing gout. For this purpose, the authors studied one of the main redox sensitive signaling cascades involved in unloading-induced atrophy, i.e., p38 MAP Kinase, along with the expression of two primary muscle specific E3 ubiquitin ligases involved in proteolysis, e.g., atrogin-1 and MuRF-1. Their findings clearly showed that HS induced a significant increase in XO activity protein expression of the antioxidant enzymes CuZn, SOD, and Catalase in skeletal muscle. The most significant finding in this paper involved the inhibition of XO with allopurinol, and significantly reduced soleus muscle atrophy along with inhibiting expression of atrogin-1 and MuRF-1, which are pivotal factors of myofibril degradation in the proteasome. As stated above, more research on this potential countermeasure is warranted.

Exercise Strategies to Counteract Muscle Atrophy during Early and Long-Term Stages of Unloading. Few studies were carried out to ascertain the mechanisms for counteracting the rapid atrophy of animal skeletal muscle as presented above. In 2006, Haddad et al. [509] performed a study to test the hypothesis that an isometric resistance training paradigm targeting the medial gastrocnemius muscle of adult rodents is effective in preventing muscle atrophy during the early stages of unloading by maintaining normal activation of the insulin receptor substrate-1 (IRS-1)/phospho-inositide-3 kinase (PI3K)/Akt signaling pathway. This pathway has been shown to simultaneously create an anabolic response while inhibiting processes that up-regulate catabolic processes involving expression of key enzymes in the ubiquitination of protein for degradation of the myofibril network. The findings of this study showed that during the 5 days of unloading: 1) absolute medial gastrocnemius muscle weight reduction occurred by 20%, but muscle weight corrected to body weight was not different from normal weight-bearing controls; 2) myofibril concentration and content were decreased; and 3) a robust isometric training program, known to induce a hypertrophy response, failed to maintain the myofibril protein content. This response occurred despite fully blunting the increases in the mRNA for atrogen-1, MURF-1, and myostatin,

e.g., sensitive gene markers that activated the catabolic state. Analyses of the IRS-1/PI3K/Akt markers indicated that abundance IRS-1 and phosphorylation state of Akt and p70S6 kinase were decreased relative to normal controlled rats, and the resistance training failed to maintain these signaling markers at normal regulatory level. These findings were insightful and suggest that to fully prevent muscle atrophy responses affecting the myofibril system (which is the primary target of atrophic stimuli) during unloading, the volume of mechanical stress must be augmented sufficiently to maintain optimal activity of the IRS-1/PI3K/Akt pathway to provide an effective anabolic stimulus for the target muscle.

Based on the above information, Adams et al. [508] undertook a study to determine if resistance training, with increased volume (3 s contractions) along with the incorporation of both static and dynamic contractile components, would be effective in preventing rapid unloading-induced atrophy. Rats were exposed to 5 days of muscle unloading via HS. During that time, one leg received electrical stimulated resistance exercise (RE) that included isometric, concentric, and eccentric contraction phases. The results of this study indicate that this combined mode RE provided an anabolic stimulus sufficient to maintain the mass and myofibril content of the trained but not the contra lateral medial gastrocnemius (MG) muscle. Relative to the contra lateral MG, the RE stimulus increased the amount of total RNA (indicative of translational capacity) as well as mRNA for several anabolic/myogenic markers such as insulin-like growth facor-1, myogenin, myoferlin, and collogen III-alpha-1 and decreased that of myostatin, a negative regulator of muscle fiber size. The combined mode RE also increased the activity of anabolic signaling intermediates such as p70S6 kinase (constituents of the IRS-1/PI3K/Akt pathway). These results indicate that a combination of static- and dynamic-mode RE of sufficient volume provides an effective stimulus to stimulate anabolic/myogenic mechanisms to counter the initial stages of unloading-induced muscle atrophy.

In the context of the above findings Dupont et al. [481] also studied the role of the IRS-1/PI3K/Akt pathway during hindlimb unloading of the soleus and fast extensor digitorim longus muscles over a span of 7, 14, and 28 days in the context of performing chronic low frequency stimulation (soleus) to maintain contractile phenotype and muscle mass. The unloaded muscles induce a down regulation of the Akt pathway and up regulation of the catabolic FOXO1 and muscle specific MURF-1, i.e., markers indicative of a catabolic state. Chronic low-level stimulation of the soleus muscle failed to maintain muscle mass at all the time points examined but did maintain the slow MHC phenotype in the soleus (e.g., non-switching of slow to fast MHC phenotype). These findings indicate the importance of loading the target muscles in order to maintain a bias of anabolic stimuli relative to the catabolic state that prevailed with the low frequency stimulation. One of the primary findings of the low frequency stimulation model is that it actually induces muscle atrophy when combined with normal loading conditions. Thus this type of countermeasure is counterproductive to maintaining muscle mass.

Does Aerobic Exercise Serve as a Protective Preconditioning Stimulus to Unloading-Induced Atrophy? Fujino et al. [527] conducted a study to determine if 25 min of aerobic treadmill running provides a protective precondition stimulus to counteract the deleterious effects of hindlimb unloading of adult male rats. The following groups were studied: a ground-based control group; a 2-week hindlimb suspension group; and a group that performed 25 min of aerobic exercise prior to undergoing 2 weeks of hindlimb suspension. The results of this study were quite surprising. As expected, soleus mass, maximum tetanic tension, myofibrillar protein content, muscle fatigue resistance, and percent of type I MHC were decreased in unloaded rats compared to the ground base control. In addition, markers for the cathepsin, calpain, caspase, and ATP-ubiquitin-proteasome proteolytic pathways were increased in the suspension group compared to the ground controls. However, the preconditioning endurance exercise bout attenuated all of the

detrimental changes associated with HS and also increased expression of heat shock protein 72. The authors concluded their findings indicate that exercise pre-conditioning may be an effective countermeasure to buffer the detrimental effects of chronic decreases in activation and loading levels on skeletal muscle, and HSP 72 may be one mechanism associated with these responses. If these findings can be verified by additional studies, this countermeasure strategy could open up a new avenue in terms of preventing the various deleterious alterations that impact animal and human skeletal muscle in unloading environments.

Role of the "Myonuclear Domain" in the Regulation of Muscle Cell Size. One of the unique features of skeletal muscle cells is that it is the only cell type that expresses multiple nuclei in each fiber cell. It has been thought that each nucleus in any given fiber manages a specific volume of cytoplasm [528]. Over the years, several studies have provided evidence that the nuclear domain is not static. Rather, during unloading conditions, myonuclei are reduced as the size of the fiber is reduced [529]**.** Also**,** the opposite occurs when the myofiber becomes hypertrophied in response to anabolic stimuli [529]. Bruusgaard et al. [528] challenge this long-standing hypothesis when they demonstrated that atrophy induced by hindlimb suspension (HS) involving adult female rats does not lead to loss of nuclei despite a strong increase in apoptotic activity of other types of nuclei within the muscle tissue (e.g., non-muscle type nuclei). Thus, in the authors' view, HS is similar to other atrophy models such as denervation, nerve impulse block, and antagonist ablation. The authors discuss several flaws concerning the different studies published to date that can be attributed to difficulties in separating myonuclei from other non-muscle nuclei surrounding the myofibers along with systematic differences in passive properties between normal and unloaded muscle. During reloading after HS, a normal re-growth was observed, which has been believed to be accompanied by recruitment of new myonuclei from satellite cells expressed outside of the fibers. However, in this study [528] the authors observed that reload led to an increase in CSA of 59%, and fiber size was completely restored to normal pre-HS size with no parallel increase in the number of myonuclei incorporated into the fibers. Thus, radial regrowth seems to differ from de novo hypertrophy in that nuclei are only added from de novo hypertrophy when muscle fibers are induced to a new and larger muscle fiber size. Clearly, these findings are important and deserve further scrutiny and follow up studies given the importance of understanding cellular mechanisms of both atrophy and hypertrophy processes.

Why are Slow Type Muscles More Sensitive to Unloading-Induced Atrophy than the Fast Type Muscles? It is apparent that slow type muscles such as the soleus, vastus intermedius, and adductor longus are more sensitive to unloading conditions such as space flight/HS than their fast type counterparts such as the plantaris and medial gastrocnemius [478]. The mechanisms impacting this differential response are essentially unknown. However, a study published in 2012 by Bortoloso et al. [530] provided some important information that may shed light on this interesting phenomenon. They studied the expression of a new and diversified family of proteins called "Homers". These Homer isoforms (e.g., 1b/c and 2a/b) were characterized in fast- and slow-twitch skeletal muscle in rats and mice. Homer 1b/c was identical irrespective of the muscle type; whereas Homer 2a/b was characteristic of the slow- twitch phenotype such as the soleus. Transition in Homer isoform was studied in two established experimental models of atrophy, i.e., after denervation and hindlimb unloading in slow twitch skeletal muscle of the rat. No change of Homer 1b/c was observed up to 14 days of denervation; whereas Homer 2a/b was found to be significantly decreased at 7 and 14 days of denervation by 70 and 90%, respectively, which paralleled the reduction in muscle mass. Seven-day HS decreased Homer 2a/b by 70%. Interestingly, reconstitution of Homer 2 by in vivo transfection of denervated soleus muscle allowed partial rescue of the atrophic phenotype, as far as muscle mass, muscle fiber size, and ubiquitination are concerned. The counteraction effects of exogenous Homer 2 were mediated by down regulation of MURF-1, Atrogen-1, and Myogenin, i.e., all genes known to be up-regulated

at the onset of atrophy. Accordingly, the present data show that 1) down regulation of Homer 2 is an early event of slow muscle atrophy and 2) Homer 2 participates in the control of ubiquitnization and ensuing proteolysis via transcriptional down regulation of MuRF1, Atrogen 1 and Myogenin. Therefore, Homers are key players of skeletal muscle plasticity, and Homer 2 is required for trophic homeostasis of slow-twitch muscle.

Newly Discovered Genes that Regulate Muscle Mass Stability and Atrophy Mechanisms. Several genes have been discovered that play a major role in determining the stability of muscle mass hoemoestasis. For example, the Scott Kimball/Leonard Jefferson group [513, 531] discovered the role of REDD1 and REDD2 genes as pivotal regulators of MTORC1 anabolic signaling pathway in the models of limb immobilization, limb suspension, and bed rest that impact muscle atrophy. Studies were performed on male rats that were subjected to unilateral hindlimb immobilization for 1, 2, 3, or 7 days or served as non-immobilized controls. Following overnight fasts, rats received either saline or L-Leucine by oral administration as a nutrient stimulus. Hindlimb skeletal muscles were processed and analyzed for the rate of protein synthesis, MRNA expression, phosphorylation state of key proteins in the mTORC1 signaling pathway, along with mTORC1 signaling repressors, REDD1/2. In the basal state mTORC1 signaling and protein synthesis were repressed within 24 hrs. in the soleus muscle of the immobilized compared to the non-immobilized hindlimb. These responses were accompanied by a concomitant induction in expression of REDD1/2. In contrast with the L-leucine stimulus, there was elevation of similar magnitude in mTORC1 stimulus in both the immobilized and non-immobilized muscle, which was accompanied in the phosphorylation of the 70-kDa ribosomal protein S6 kinase in only the nutrient stimulus group. These findings suggest that signaling through mTORC1 becomes impaired in response to immobilization by induction of REDD1/2 causing a defective of the p70S6 kinase enzyme.

In a follow up study [513], the authors studied the mechanism of why immobilized skeletal muscle fixed in a shortened position displays disuse atrophy, whereas it does not atrophy when fixed in a stretched position. They tested the hypothesis that skeletal muscle in the stretch position would be protected from gene expression changes known to be associated with disuse atrophy such as REDD1/2. To test this hypothesis, male rats were subjected to unilateral hindlimb immobilization for 3 days with the soleus fixed in either a shortened or stretched position with results compared to the contra lateral non immobilized muscle. Soleus immobilized in a shortened position exhibited disuse atrophy, attenuated rates of protein synthesis, attenuated mTORC11 signaling, and induced expression of genes encoding REDD1, REDD2, Atrogin-1 and MuRF1 (markers of protein degradation). In contrast, immobilization in the stretched position prevented these changes as it exhibited no difference in muscle mass, rates of protein synthesis, mTORC1 signaling, or expression of genes encoding REDD1, REDD2, Atrogin-1 and MuRF1. Thus muscle immobilized in the non-stretched position leads to induction of gene expression for REDD1, REDD2, and the atrogenes that induce protein degradation.

Complementing the findings presented above, Nakao et al. [532] reported that skeletal muscle atrophy caused by unloading is characterized by both a decreased responsiveness to myogenic growth factors (e.g., insulin-like growth factor 1 (IGF-1)) and increased proteolysis. This occurs via the induction and activation of the ubiquitin ligase, Cbl-b. Upon induction, Cbl-b interacts with and degrades the IGF-1 signaling intermediate IRS-1. In turn, the loss of IRS-1 activates the FOXO3-dependent induction of atrogen-1/MAFbx, a dominant mediator of proteolysis in atrophying muscle. Cbl-deficient mice were resistant to unloading-induced atrophy and the loss of muscle function. Furthermore, a pentapeptide mimetic of tyrosine (608)-phosphorylated IRA-1 inhibited Cbl-b mediated IRS-1 ubiquitination and strongly decreased the Cbl-b-mediated induction of atrogen-1/MAFbx. These observations indicate that the Cbl-b-

dependent destruction of IRS-1 is a critical dual mediator of both increased protein degradation and a reduced protein synthesis observed in unloading-induced muscle atrophy. Also, the inhibition of Cbl-b mediated ubiquitination may be a new therapeutic strategy for treating unloading-mediated muscle atrophy.

Novel Insights on Isolated C2C12 Myocytes in Models of Atrophy. A study by Kazi et al. [533]**,** using isolated C2C12 myocytes in cell culture, has provided very interesting results concerning the role that Deptor plays in protein metabolism. Deptor is an mTOR binding protein thought to inhibit mTOR-S6Kinase signaling during protein synthesis. The authors postulated that by knocking down Deptor expression in C2C12 myocytes, mTOR activity and protein synthesis would occur. Deptor knockdown was achieved by using lentiviral particles containing short hairpin (sh) RNA targeting the mouse Deptor mRNA sequence. Knockdown reduced Deptor mRNA and protein content by 90%, which increased phosphorylation of mTOR kinase substrates, 4E-binding protein-1 and S6Kinase1, and concomitantly increased protein synthesis along with causing larger cell size. Interestingly, Deptor knockdown (50% reduction) by electroporation into gastrocnemius of C57/BL6 mice did not alter weight or protein synthesis in control muscle. However, Deptor knockdown prevented atrophy by 3 days of hindlimb immobilization by increasing protein synthesis. These findings support the notion that Deptor is an important regulator of protein metabolism in myocytes and demonstrate that decreasing Deptor expression in vivo is sufficient to ameliorate muscle atrophy.

Is Loss of Skeletal Muscle Mass and Function Experienced by Astronauts and Animals during Space Flight Impacted by Ionizing Radiation? It is unknown whether loss of skeletal muscle and functioned experienced by astronauts during space flight could be augmented by ionizing radiation (IR), such as low-dose high-charge and energy (HZE) particles of low-dose high-energy proton radiation. Shtifman et al. [534] performed a study on adult mice that were irradiated whole body with either a single dose of 15cGy of 1GeV/n Fe particles or with a 90cGy proton of 1GeV/n proton particles. Both ionizing radiation types caused alterations in the skeletal muscle cytoplasm Calcium- 2 (Ca2) homeostasis. Fe-particle irradiation also caused a reduction of depolarization-evoked Ca2 release from the sarcoplasmic reticulum. The increase in the calcium content was detected as early as 24 hrs after Fe-particle irradiation, while effects of proton irradiation were only evident at 72 hrs. In both instances, calcium content returned to base line at day 7 after irradiation. Neither un-irradiated controls nor proton-irradiated samples exhibited such a phenotype. Protein analysis revealed a significant increase in the phosphorylation of AKt, Erk1/2, and rpS6K on day 7 in Fe-particle irradiated skeletal muscle, but no increase was revealed in proton or un-irradiated skeletal muscle, suggesting activation of pro-survival signaling. These findings suggest that a single low-dose Fe-particle or proton exposure is sufficient to affect Ca2 homeostasis in skeletal muscle. However, only Fe-particle irradiation led to the appearance of central nuclei and activation of pro-survival pathways, suggesting an ongoing muscle damage/recovery process. These findings beg the issue of what a chronic exposure would do to the steady state health of the muscle system.

q. Summary of Animal Experiments

The use of animal research models during space flight and in space flight analogs has been an invaluable tool in better understanding the effects of unloading-induced skeletal muscle adaptation. Animal research has corroborated a number of human space flight findings observed in far more limited cohorts, thus giving support to the physiological models. For example, animal research has shown that space flight primarily affects postural muscles, larger fibers are generally more susceptible to muscle atrophy than are smaller fibers, and that slow fibers are more affected than are fast fibers. Animal research has also been at the cutting edge of our understanding of

how space flight negatively affects muscle mass. Animal models have been used to identify the key molecular pathways that regulate muscle protein synthesis as well as protein degradation in mammalian skeletal muscle. Animal models have also shown where there are lesions in the normal regulation of theses pathways in response to space flight or space flight analogs. This information may be vital in moving forward in developing effective new countermeasures that directly target the regulators of muscle mass most affected by space flight and in understanding why other countermeasures may fall short. Animal research also plays an important role in the comprehensive study of the effects of space flight on skeletal muscle by providing a model for which human experiments cannot be directly tested (e.g., space radiation studies). In short, animal space flight research has provided both corroborating and leading-edge scientific knowledge base needed to adequately mitigate the effects of reduced muscle mass, strength, and endurance in skeletal muscle.

r. Computer-Based Models

i) Aerobic Capacity

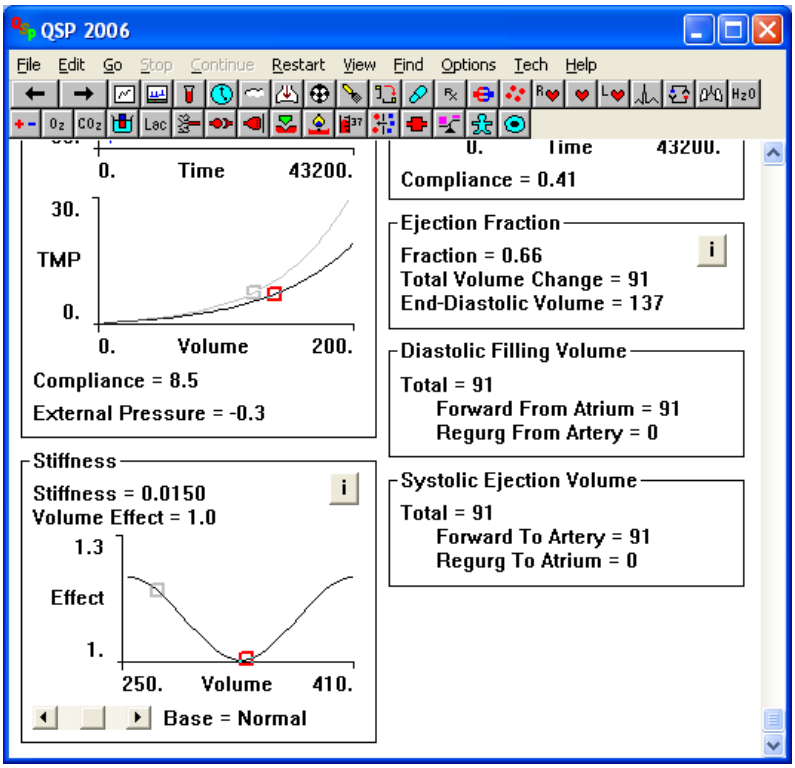

***Figure 55**. Simulation of microgravity effects on plasma volume and diastolic function.*

Since VO_2peak is primarily determined by Q_c, we would expect any factors related to heart function or plasma volume to be of functional significance. The impact of the microgravity induced changes in both plasma volume and diastolic function are integrated into the operation of the Digital Astronaut as noted in the graphic below (Figure 55). The upper left-hand curve in the panel describes the diastolic compliance of the left ventricle as it relates transmural pressures (TMP) to ventricular volumes. The lighter curve, as indicated by the arrow, depicts the shift in the

compliance curve upon adaptation to the microgravity environment. This shift is due to the stiffness changes that occur with the fluid shifts in microgravity and a relative dehydration of the ventricular interstitial spaces. The stiffness of the left ventricle as a function of the interstitial fluid volume was described by Pogatsa [535] and is shown in the curve in the lower part of the panel. Simulation studies using the Digital Astronaut Model replicate the findings demonstrated by Levine et al. [42] of a 10% decrement in VO_2peak upon reentry. This performance validation of the model predictions allows us to extrapolate what might be expected for VO_2peak changes immediately upon entering a Mars or lunar gravitational field (Figures 56 and 57).

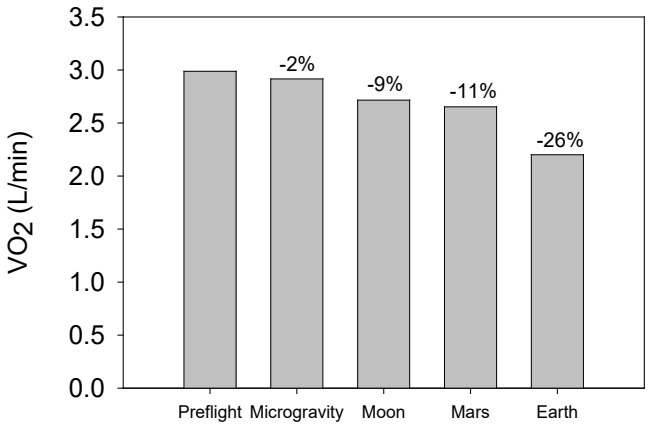

Figure 56. Model prediction of various gravity profiles on VO_2peak.

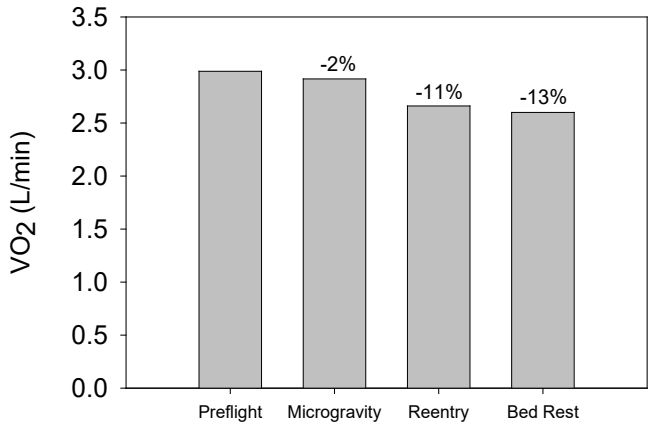

Figure 57. Simulation of the effects of spaceflight on VO_2peak

ii) Strength

Utilizing computational modeling to predict the influence of microgravity or the efficacy of countermeasures on skeletal muscle mass and function was developed by NASA under the Digital Astronaut Project (DAP) [536]. The goal of the DAP is to develop and implement well-validated computational models to predict and assess space flight health and performance risks and to enhance countermeasure development. To ensure the computational models appropriately represent the physiologic process that may play a role in space flight, the DAP works closely with NASA's subject matter experts in muscle and exercise physiology. Given the early stage of this work, peer-reviewed citations do not exist regarding the use of these models in predicting the loss of skeletal muscle mass and function in a microgravity environment or to predict the efficacy of exercise countermeasures.

2. RISK IN CONTEXT OF EXPLORTATION OPERATIONAL SCENARIOS

The principal risks of reduced VO_2peak and muscle strength from the deconditioning of prolonged 0g exposure is the inability for crewmembers to perform critical tasks to meet mission objectives. The following explorational scenarios with prolonged partial gravity exposure are impacted from reduced aerobic capacity and muscle strength and endurance:

- Completing necessary EVA tasks (during space flight, as well as on Lunar or Martian surfaces)
- Completing nominal or emergency egress scenarios upon return to Earth, Lunar or Martian surfaces

It is important to note that the exploration operational risk(s) related to loss of aerobic capacity, skeletal muscle mass, strength, and endurance influence the successful completion of mission critical tasks, which are affected by 1) the relative and 2) the absolute loss from preflight, as significant decrements in relation to crew's maximal capacity may impact the physiological expense required to successfully complete a requisite set of tasks within a fixed or prolonged period of time. Thus, a crewmember must be capable of completing a task before being exposed to microgravity or partial gravity, with preflight fitness that can accommodate the amount of functional loss expected with space flight. However, decrements in aerobic and muscular fitness cannot be allowed to fall below the level needed to successfully complete all assigned tasks. Therefore, the physical performance requirements for completion of the tasks should be known. Without information relating to the physical performance requirements of tasks, it is not possible to determine the risk of failure. Additionally, if a task could not be completed by a crewmember before microgravity or partial gravity exposure, it can reasonably be stated that the risk of failure during a mission is 100%. Additionally, even if the crewmember has the capability to complete every possible individual task, a composite of the tasks to be completed over a prolonged period of time presents an entirely different requirement. Further, all possible contingencies that might arise must be considered so that a crewmember will be able to manage such off-nominal scenarios even near the end of a duty day. Thus, even an approach as basic as thoughtful scheduling of daily tasks and incorporating rest and work ratios could serve to help mitigate risk. Several important items must be known with respect to the risks related to loss of VO_2peak, skeletal muscle strength, and endurance. These include:

- Physiological demand of a task or set of tasks to be completed
    - Absolute and relative to maximal capacity (metabolic rate, oxygen cost)
    - The time period in which the tasks need to be performed
- Clear understanding of the Notional Design Reference EVA
- Baseline preflight level of crewmember functional performance
    - Aerobic capacity, strength, endurance, and functional fitness
- Expected and actual magnitude of functional loss from baseline at any point during the mission
- All possible credible contingency events that could have an impact on functional performance
- Any other interfering conditions that could affect functional performance
    - Planetary g (Earth, Lunar, or Martian surfaces)
    - Nutritional and psychological status
    - EVA suit specifications (thermal, CO_2, mass and design)
    - Equipment malfunction or failure, illness, injury, etc.
- Hardware and exercise prescription countermeasures needed to maintain aerobic capacity and muscel strength and endurance

Physiological demand of a task or set of tasks to be completed. During Lunar EVAs conducted during the Apollo era, EVA intensities were up to ~85% of maximum HR during some tasks [71]. These crewmembers were cautioned to slow their activities. However, in the future, oxygen uptake associated with building structures or accomplishing more strenuous tasks during longer stays on the Moon will likely be as great as or greater than that encountered during Apollo.

Furthermore, there is a risk associated with emergency egress upon return to Earth or in a partial gravity environment, where maximum aerobic efforts may be required under high stress conditions [70].

The metabolic cost of EVAs in microgravity based on Shuttle, Skylab, and Apollo missions [537] reported 196 ± 34 kcal/hr (energy expenditure) or 666 ± 117 mL/min (oxygen cost, VO2; assuming 4.9 kcal per 1 L of O_2) for prolonged periods of time (3–9 hrs; mean±SD, 5.8±1.6 hrs). Depending on the crew's preflight aerobic capacity and in-flight reduced fitness (10–20% loss), assumptions can be made on successful EVA and level of effort relative to crew's aerobic capacity. For example, crew with low absolute aerobic capacities will work at a higher relative intensity compared to crew with high aerobic capacities. Table 16 shows estimated work capabilities of EVAs relative to a fraction of aerobic capacity that is dependent on preflight fitness and absolute aerobic capacity. Moreover, a 10–20% loss in-flight will require a higher level of effort (fraction of VO_2peak). Current minimal standards (NASA-STD-3001) report preflight aerobic capacity at 32.9 ml/kg/min for an 82kg person, the absolute VO_2peak is 2624 mL/min.

Table 16. *Estimated oxygen cost of microgravity EVAs for low, mean, and high absolute aerobic capacity.*

	Preflight		In-flight			
	ml/min	Fractional cost	10% loss	Fractional cost	20% loss	Fractional cost
Low	1900	35%	1710	39%	1368	49%
Mean	3100	21%	2790	24%	2232	30%
High	5500	12%	4950	13%	3960	17%

EVA cost 666 mL/min

Low, mean, and high represents near lowest, mean, and near highest recorded preflight ISS VO_2peak, Fractional cost of work (est. at 666 mL/min) is the percentage of preflight VO_2peak.

There were reported metabolic costs that were of higher physical intensities for shorter time periods of about 23 min (300–350 Kcal/hr or 1020–1191 mL/min), 6 min (350–400 Kcal/hr 1191–1361 mL/min), and 5 min (>400 Kcal/hr or >1361 mL/min) [537]. Table 16 shows the estimated cost of work of 1000 ml/min for about 20 min is near 50% VO_2peak of crew that have low absolute aerobic capacity (1900 mL/min) and less taxing for the mean (~30% VO_2peak) and high (20% VO_2peak) aerobic capacity crews. With 10–20% loss in aerobic capacity, the fractional utilization of this work increases to 60–70% VO_2peak for low absolute aerobic capacity crew. For the mean group, this increases to 36–45% VO_2peak and to 20–25% VO_2peak for the high aerobic capacity crew. The higher recorded workloads of 1400 ml/min for the low aerobic capacity for 5 min becomes very exhausting at 74% VO_2peak; however, the assumed reduced loss of 10–20% would make performing high workloads near impossible, as low aerobic capacity crew would be at 82–102% VO_2peak. For the mean (45%) and high (25%) aerobic capacity crews, the fractional utilization further increases and, with estimated loss of 10–15%, becomes more taxing (50–60% and 28–35% VO_2peak).

It is important to note that intensities above ventilatory threshold will increase reliance of glycolytic metabolism that result in elevated metabolic byproducts such as CO_2 and metabolic heat production. These byproducts are expelled into the closed spacesuit environment via ventilation and heat dissipation from the skin surface through heat exchange pathways (i.e., radiation, conduction, evaporative heat loss) that will cause an increased risk of fatigue, hypercapnia, and thermal load in the suit. We have reported that this occurs at 55–67% of crew VO_2peak [149].

Table 17. *Estimated metabolic and oxygen cost of microgravity EVAs for low, mean, and high absolute aerobic capacity.*

	Preflight		In-flight			
	mL/min	Fractional cost	10% loss (mL/min)	Fractional cost	20% loss (mL/min)	Fractional cost
Low	1900	53%	1710	58%	1368	73%
Mean	3100	32%	2790	36%	2232	45%
High	5500	18%	4950	20%	3960	25%
EVA cost 1000 mL/min						
Low	1900	63%	1710	70%	1368	88%
Mean	3100	39%	2790	43%	2232	54%
High	5500	22%	4950	24%	3960	30%
EVA cost 1200 mL/min						
Low	1900	74%	1710	82%	1368	102%
Mean	3100	45%	2790	50%	2232	63%
High	5500	25%	4950	28%	3960	35%
EVA cost 1400 mL/min						

Low, mean, and high represents near lowest, mean, and near highest recorded preflight ISS VO_2peak, Fractional cost of work (est. at 1000, 1200, and 1400 mL/min) is the percentage of preflight VO_2peak and with the assumptions of 10 to 20% loss.

EVAs on the Lunar and Martian surface will introduce an additional 0.17 g and 0.38 g environmental influence that need to be factored in with the mass of the crew and suit as well as the loss in fitness. Analog studies have reported ambulation in a pressurized suit in Lunar and Mars gravity to be a physical demanding task [538]. Notional xEMU mass is between 400–440lbs. EVAs in Lunar and Martian gravity will increase metabolic rates that will be dependent on crew and suit mass and fitness. Additionally, portable life-support system (PLSS) useful for life on the

Lunar and Martian surface will be dependent on the usage rate of consumables (i.e., oxygen, sublimator water supply, and carbon dioxide absorber).

Table 18. *Impact of aerobic fitness, body and suit weight, and gravity on estimated metabolic and oxygen cost of Lunar and Martian surface EVAs*

	Preflight Earth 1 g				Lunar (0.17 g) oxygen cost of total weight				Martian (0.38 g) oxygen cost of total weight			
	VO_2peak (mL/min)	crew mass (kg)	suit weight (kg)	total weight (kg)	Lunar total weight (kg)	mL/min	mL/kg /min	Fractional cost (%)	Mars total weight (kg)	mL/min	mL/kg /min	Fractional cost (%)
Low	1900	60	204	264	45	1000	17	53%	100	2236	37	118%
Mean	3100	80	204	284	48	1076	13	35%	108	2405	30	78%
High	5500	95	204	299	51	1133	12	21%	114	2532	27	46%

Note: Estimated metabolic work assumption based on linear relationship of work rate and weight (7.6 W per 1 kg load [48]). Assumptions 1kcal/min = 70W [50] and 4.9kcal/min = 1LO_2 Lunar and Martian surface EVAs total mass = crew body weight + spacesuit weight × 0.17 (Lunar g) or 0.38 (Martian g). Relative VO_2peak to body weight for low, mean, and high are 31.7, 38.8, 57.9 mL/kg/min.

Estimations of Lunar surface metabolic rates for Apollo missions were reported using an integration of three methods (heart rate, oxygen, and liquid cooling garment methods) and determined an estimated 10–15% variability [537]. EVA duration on Lunar surface was (mean±SD) 5.6 ± 1.8 hr and consisted of Apollo Lunar Surface Experiments Package deployment (240±50 Kcal/hr or 832±173 mL/min), geological station activity (251±41 Kcal/hr or 870±141 mL/min), overhead (267±35 Kcal/hr or 928±120 mL/min), and Lunar rover vehicle operations (235±28 Kcal/hr or 816 ±93 mL/min). Because the weight of the crew and spacesuit will have more impact on oxygen cost, normalization to kg weight is also estimated to be on average for all activities 11±1 mL/kg/min (Table 18).

Table 19. *Metabolic and oxygen cost of Lunar surface EVAs from Apollo*

	ALSEP deployment	Geological station activity	Overhead	Lunar roving vehicle operations	Mean for all activities
kcal/hr	240±50	251±41	267±35	124±22	249±28
mL/min	815±170	852±138	909±118	421±74	845±94
mL/kg/min	11±2	11±2	12±2	6±1	11±1

Note: Apollo mission 11–17 body mass of crew were 75±3 kg [29], ALSEP, Apollo Lunar Surface Experiments Package deployment. Data from [36] conversion of KJ/h = 0.239 kcal /h, 4.9kcal = 1 L O_2

Table 19 shows estimated crew mass and suit on Lunar and Martian surface, and the impact on metabolic rates in low, mean, and high aerobic capacity crews. Notably, the mean and high estimated oxygen cost values are within the mean and deviation of Apollo data in Table 19. Because the mass of the suit may not differ among crew, smaller crew and those with lower aerobic capacity will require a higher fractional utilization of their aerobic capacity (53% VO_2peak) on the Lunar surface. These estimates for long-duration EVAs are reported as averages and do not show the possible shorter burst of very high activity as reported in Table 16. Moreover, of

greater concern in this estimated scenario, the low aerobic capacity crew will exceed their aerobic capacity limits (118% VO_2peak) on the Martian surface. The mean aerobic capacity crew is estimated to use 35% VO_2peak on the Lunar surface, and comparable work on the Martian surface would be considered exhaustive at 78% VO_2peak. Notably, crew with high aerobic capacity will be working at lower fractional cost on the Lunar (21% VO_2peak) and Martian surface (46% VO_2peak). It is of high importance to maintain crew health and performance. A 10–20% reduced aerobic capacity would make it nearly impossible to complete EVA tasks on the Martian surface for prolonged periods of time for crew with low and mean aerobic capacities and may also require short bursts of high intensity activity.

Return to Earth nominal and emergency egress scenarios are also critical upon return from low Earth orbit. Previous launch and entry suit masses worn during landing and egress procedures were 51 lb (23 kg). During an emergency bailout, an additional 26 lb (12 kg) is worn. Moreover, for an expedited contingency landing, crew are expected to lift an inflatable slide that weighs 45 lb (20 kg) against a side hatch and lock into place. Crew may also be expected to climb and exit through a top window [539]. Alexander et al. [115] reported that prediction time of emergency capsule egress on a custom-built mock-up of the NASA Orion capsule time was about 1 min. The egress protocol was to move from the seat to the left, move two 5-kg bags about 1 m to a marked location, and then release and attach a rope ladder to the floor of the capsule. The subjects then carried the two packages through the top hatch and exited the capsule through the top hatch. The relative cost of this capsule egress work was 72 ± 25% VO_2peak (aerobic capacity of 24 ± 5 ml/kg/min, 2160 mL/min). Though this study provided useful data on metabolic cost of capsule egress, spaceflight deconditioning, the addition of the 23 kg suit, and lifting the inflatable slide 20 kg would increase time and metabolic cost to greater than 72% VO_2peak.

Notional Design Reference EVA. The longest cumulative time of Lunar surface EVA by a crew during the Apollo Program was about 22 hrs (combined from 3 separate days), and the longest total duration of the crew on the lunar surface was about 75 hrs during the sixth and final Apollo mission (Apollo 17). Recent EVA Conops do not plan more than 24 hrs of EVA per person per week for Artemis Lunar surface missions [39]. Table 20 shows the Notional Design Reference EVAs of 6 hr EVAs for walking of up to 2 km away and on 20° slope terrains. It will be important

Table 20*. Notional Design Reference EVA Series for xEVA Con Ops Development*

Surface day 1	Surface day 2	Surface day 3	Surface day 4	Surface day 5	Surface day 6	Surface day 7
• Landing on lunar surface • Road to EVA: xEVA system, lander, and EVA prep	• EVA 1 • 6 hr (egress to ingress) • EVA tasks (notional): contingency sample, public affairs, experiment package deploy sample acquisition • Transverse stay relatively close to the lander	• EVA 2 • 6 hr (egress to ingress) • EVA tasks (notional): characterize PSRs, acquire samples from PSRs • Traverses extend further from lander, walking up to 2 km away up/down slopes of up to 20°	• Day off from EVA	• EVA 3 • 6 hr (egress to ingress) • EVA tasks (notional): acquire samples from Ejecta Blanket • Traverses extend further from lander, walking up to 2 km away up/down slopes of up to 20°	• EVA 4 • 6 hr (egress to ingress) • EVA tasks (notional): Deploy geotechnical instrument, deploy environmental monitoring station for ISRU • Traverses extend further from lander, walking up to 2 km away up/down slopes of up to 20°	• EVA 5 • 1 hr (egress to ingress) • Prep for ascent (configure suit to VLM) • EVA tasks (notional): Jettison hardware • Ascent from surface to Gateway/Orion

Table from Exploration EVA System Concept of Operations Summary for Artemis Phase 1 Lunar Surface Mission [39]

to have a better understanding of Notional Design Reference EVAs to ensure the entire crew is capable of successfully completing long-duration EVAs and to determine the expected metabolic cost of activities.

Baseline preflight level of crewmember functional performance and expected deconditioning during prolonged spaceflight. Based on these estimates provided on Tables 16–18, the minimal aerobic capacity threshold requirement (NSAS-STD 3001 [30]) of 32.9 mL/kg/min on ISS may need careful review for the development of standards for planetary exploration missions, as physical work and deconditioning will reach near maximal aerobic capabilities on the Lunar and Martian surfaces. The metabolic cost of EVAs and emergency egress scenarios are highly dependent on deconditioning and the mass of crew and spacesuits. Future work should use simulations walking in 0.17 and 0.38 g and evaluations of variations of low, mean, and high aerobic fitness and strength. Moreover, it is unknown how strength loss may contribute to EVA performance.

Prolonged exposure to 0g reduces VO_2peak rapidly over short-duration and progressively decreases over longer duration with no exercise (Figures 41 and 44). Exercise countermeasures incorporating various prescriptions and hardware have been shown to protect crew from deconditioning; however, these countermeasures are not fully protective for all crew. Moreover, the large variability of changes from pre to postflight suggest that there are some crewmembers who have greater than 10–20% reductions in aerobic capacity (Figure 21) and muscle strength and endurance (Figures 26 and 27), increasing risks to exploration operational scenarios. Additionally, short burst of high intensity activity over various slopes and terrain are likely scenarios. Notional Design Reference EVA Series for xEVA Con Ops Development have been developed with expectations to walk 2 km away from lander and of 20° slopes (Table 20). Completing explorational EVA tasks are further complicated by sensorimotor impairments.

The ISS NASA-STD 3001 aerobic capacity standards report that countermeasures shall maintain in-flight skeletal muscle strength at or above 80% of preflight values [30]. However, as estimated in Tables 17 and 18, a 20% loss in aerobic capacity will require higher fractional utilization of crew aerobic capacity, especially for low and average aerobic capacity. In addition, the assumption that activities requiring a very high burst of intensity reported in Apollo missions will increase usage rate of consumables (i.e., oxygen, sublimator water supply, and carbon dioxide absorber) and risks of premature fatigue, hyperthermia, hypothermia, and hypercapnia. It is imperative that all crew maintain health and performance for exploration missions. For future Artemis missions to the Lunar surface, exploration vehicles will have limited volume and will not have the similar hardware capabilities as ISS. This may alter the effectiveness of hardware to provide adequate stress on the body to maintain strength and bone density. Newer, more compact exercise hardware is currently being evaluated. This includes the European Enhanced Exploration Exercise Device (E4D) [540] and Orion Flywheel device. The E4D is currently in development and has not been evaluated for exercise responses. Metabolic rate, oxygen uptake, heart rate, work relationships, and force plate load profiles are needed to assess the stress of the hardware on the human body. Moreover, exercise responses on the hardware need careful evaluation on the chronic adaptations. Lastly, in-flight evaluation of hardware exercise response may differ in 0 g compared to 1 g. The Flywheel device has been studied in a 60-day head down bed rest study in females with lower body negative press (LBNP), which reported that prescription of LBNP for 3–4 days and Flywheel exercise for 2–3 days has less of a reduction for lumbar paraspinal muscle loss (−4%) compared to no exercise control (−14%) [541]. Additionally, total BMD loss and VO_2peak were less or maintained compared to the no exercise control. However, isokinetic strength was reduced by 30% in both the LBNP + Flywheel and control no exercise

groups, suggesting lack of strength adaptations using Flywheel device [542]. However, this may be due to the exercise prescriptions. The Flywheel device was used in a 70-day head down bed rest study and found similar exercise adaptations for bone markers, VO_2peak, and parameters of muscle performance (leg press total work, isokinetic upper and lower leg strength, vertical jump power, and maximal jump height, as well as muscle size) compared to traditional resistance exercise [441]. Exercise prescription for the Flywheel device was of higher intensity for resistance exercise 3 days/wk using a nonlinear periodized model. Future exploration exercise hardware will need careful evaluation in-flight for exercise responses and appropriate dose of exercise needed to maintain crew health and performance.

Regarding the question of whether exercise equipment should be available to crewmembers for short missions to the Moon and back, the answer is easy: a resounding "yes." During some of the Apollo missions, a small, lightweight device called the "Exer-Genie," which required no external power, was made available to crewmembers (see Figure 9), and they were encouraged to use it. Specific comments from the Apollo crewmembers collected during the 2007 "Apollo Summit" are particularly relevant [543] and can be summarized as follows:

- Apollo crewmembers felt that crew surgeons and mission planners should not hard-schedule exercise prescriptions for such short-duration missions, but the crew should be allowed to perform them at their leisure.
- They stated that a more robust and lightweight piece of in-flight exercise equipment is needed than what was flown during Apollo. The Exer-Genie was limited, its ropes were friable, and the device generated a lot of heat and smell; therefore, an alternative exercise device is needed.
- Most crewmembers felt that the pre-mission timeline should provide adequate time to maintain musculoskeletal strength and stamina. Some astronauts attributed their capabilities on the Lunar suface to pre-mission training because in some cases more force was needed on the Lunar surface while wearing the EVA suit than was needed in 1 g on Earth.
- The crew felt that Exer-Genie or an alternative was definitely needed, and because of a fear that they would break it, they actually tapered off from intense use to save it for use in reconditioning on the return trip before re-entry.
- The strongest comment was that "as many exercise capabilities as possible should be built into all future vehicles" because they will get used, and the crew further felt that exercise capability throughout flight was critical and that a variety of exercises should be provided.

Lunar outpost missions may present a greater challenge than shorter "sortie" missions, but with respect to the current risk topic, they probably represent risks similar to those experienced on the ISS. Lunar gravity, although about 1/6 that of Earth gravity, may provide more loading to maintain muscle mass and function than microgravity, but currently this question remains unanswered and in the context of requirements for robust hardware capabilities and exercise prescriptions needed to maintain crew health and performance. Certainly, exercise regimens and hardware will be required, not only for countering reduced VO_2peak and muscle strength but for the reasons stated by Apollo astronauts above. How much exercise is needed and the proper way to implement it are certainly knowledge gaps that require innovative research to fill. Part of this research will unquestionably help to define the level of risks to which crews will be exposed, but it will also be helpful in properly mitigating those risks.

Artemis mission II is scheduled to fly the first scheduled crew mission on Orion spacecraft in May 2024 and perform a Lunar flyby test and return to Earth. The total mission duration is scheduled to be 11 days. Artemis mission III will be the first scheduled crew mission Lunar landing with the total mission duration scheduled to be 28–34 days, with 11 days docked on Orion and 6.5 days crew surface days [327, 544]. There will be about 16 days of in-flight exposure to microgravity before reaching the Lunar surface (partial gravity) where deconditioning may occur if appropriate exercise countermeasures are not provided to crew. Furthermore, the smaller volume vehicle of Orion and Gateway place limitations on exercise hardware design and capability and exercise prescriptions needed to provide adequate exercise stress to prevent deconditioning.

Without doubt, transport between the Earth and Mars, as well as the return trip, represent the greatest risks to humans encountered in the history of human space flight. Notwithstanding, the risks of radiation exposure and the deterioration of the musculoskeletal system must be prevented or a mission to Mars (and back) will not be successful. Highly refined exercise protocols and robust exercise equipment and methods to monitor functional capacity are mandatory for mitigation of the risks inherent in long-duration exposure of humans to microgravity. A huge challenge will be to provide the above within the current designs of different feasible Mars transit vehicles. The cramped confines will afford little room for stretching or exercise and the larger volume exercise hardware comparable to ISS. Modest power (or in some instances, no power) available for equipment and a human life support system, whose design may be marginal to support a full complement of exercise by efficiently dealing with the heat, water vapor, and carbon dioxide that are byproducts of human exercise, is an additional challenge that must be overcome.

Knowledge gained during Lunar outpost missions will be highly relevant to the successful establishment of a Martian outpost. If the challenges posed by the long transit to Mars and the extended period of microgravity exposure can be met, the outpost phase should represent a much lower risk by comparison, since Lunar outpost experience will have allowed significant opportunity to develop risk-mitigation strategies for this phase. The gravitational environments are similar; in fact, the Martian gravity field, being greater than that of the Moon, will provide a greater physiological stimulus to the crewmember which should complement expected exercise countermeasures. However, capability to provide sufficient exercise capacity during the Martian outpost phase is essential in preparing the crew for a long-duration exposure to microgravity on the transit back to Earth. This probably represents the greatest challenge with respect to maintaining a safe level of skeletal muscle performance for exploration-class missions, and contingency Earth landing problems are significant unknowns for the Mars mission.

a. Countermeasures and effectiveness in mitigating risk

Current ISS countermeasures and effectiveness at mitigating aerobic capacity and muscle strength and endurance risks are thoroughly described in section I of this report. The summary for aerobic capacity and leg muscle strength and endurance are listed Section 1 and 4 show that current countermeasures are not fully protective and find high interindividual variability among crewmembers for long-duration ISS missions in microgravity environments. As described in Section 4, subsection b, these risks in the context of exploration missions to the Lunar and Martian surface may be entirely different and need careful review. Current DRMs and spacesuit capabilities are unknown and are needed to make appropriate assumptions on the capability of crew to perform successful exploration EVAs. Understanding the variability of exercise response for maintaining aerobic capacity and muscle strength and endurance will be important for all crew to mitigate risks.

b. Operational perspective on the risk with emphasis on changes in procedures/standards/requirements on spacecraft

As described above, ISS countermeasures are not fully protective, and high interindividual variability are current unknowns. From an operational perspective, understanding the exercise prescriptions and developing standards may reduce the large individual variability. For exploration missions, current unknowns of the DRMs make it difficult to address this. However, it must be assumed that current ISS exercise requirements and standards for microgravity may not be the same for exploration missions with less capable hardware. As described in section 4, subsection b, the current gaps in knowledge and future work will assist in improving operational procedures, standards, and requirements on spacecrafts. At the time of this evidence report update, we suggest all standards need careful review and updates need to be specific to microgravity, Lunar, and Martian surface risks.

3. DAG REVIEW AND INTEGRATION WITH OTHER RISKS

a. DAG Review

Review of currently accepted DAG and Level of Evidence assessment of each arrow (relationships), as supported by evidence presented in the report. Changes to this section should be done shortly after DAG updates are provided (this requires coordination with HSRB).

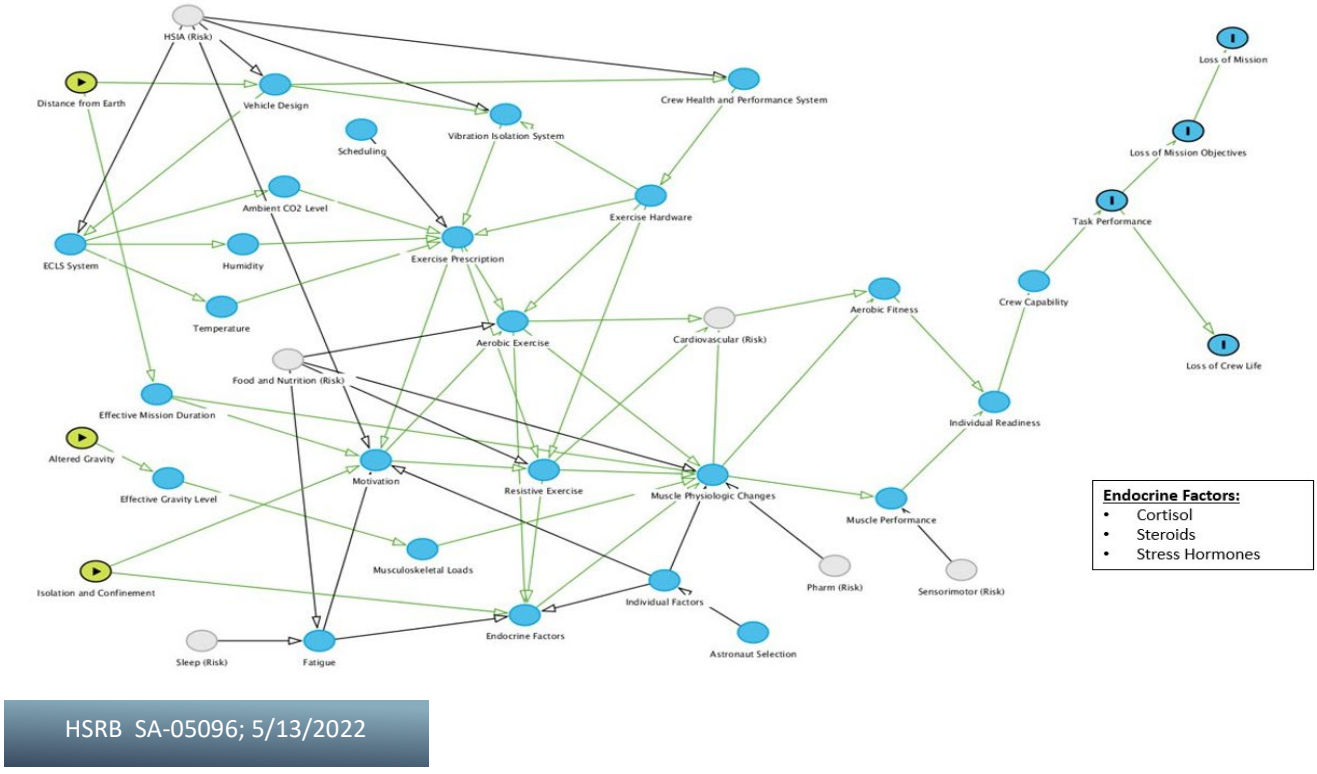

Figure 58. *Risk of Impaired Performance Due to Reduced Muscle Size, Strength, and Endurance (Muscle Risk) and Risk of Reduced Physical Performance Capabilities Due to Reduced Aerobic Capacity (Aerobic Risk)*

Muscle and Aerobic Risks DAG Narrative

- Altered Gravity across all DRMs includes microgravity, Lunar gravity, Mars gravity, and Earth Gravity. This affects Effective Gravity Level experienced by crew and the Musculoskeletal Loads.
- Isolation and Confinement, especially over long Effective Mission Durations, can induce monotony and anhedonia affecting the Motivation to perform needed exercise.
- Distance from Earth determines the mass and volume allocations for Vehicle Design and Suit Design and affects the Effective Mission Duration.
- The central focus of the Muscle and Aerobic Risk DAG is on Aerobic Fitness and Muscle Performance levels being adequate for crew to perform tasks are expected of them (Task Performance). These directly influence Individual Readiness and crew readiness or fitness for duty (Crew Capability).
- When these loads are negatively affected, the performance of EVAs—EVA (Risk)—and the performance of post-landing tasks—Crew Egress (Risk)—may be threatened.
- Aerobic Fitness and Muscle Performance are directly affected by the Muscle Physiologic Changes that occur at the level of cardiac, smooth, and skeletal muscle throughout the body as a result of the duration of exposure to the spaceflight environment. Aerobic Fitness is also directly dependent on the functionality of the Cardiovascular system (Cardiovascular Risk).
- These Physiologic Changes are the result of several contributing factors:
    - Musculoskeletal Unloading that occurs as a result of the Altered Gravity Environment
    - Endocrine Factors that are related to the Isolation and Confinement experienced
- Countermeasures to these include:
    - Resistive Exercise
    - Aerobic Exercise
    - Medications—Pharm (Risk)
    - Nutrients—Food and Nutrition (Risk)
- Other contributing factors include the Motivation to exercise which is impacted by Fatigue through the Sleep (Risk), Food and Nutrition (Risk), Effective Mission Duration that can induce monotony, and design and maintenance challenges from the HSIA (Risk).
- The exercise capability that protects Aerobic Fitness and Muscle Performance is the result of the Vehicle Design process, the HSIA (Risk), and the fielded Crew Health and Performance System. These define and limit the presence and reliability of the Exercise Hardware available on a mission. The Exercise Prescription that can be accomplished by crew is limited by the Schedule, Vibration Isolation System, Previous Injury, and environmental factors such as Temperature, Humidity, and CO2 (Risk) that are functions of the ECLS System.

b. Integration with other risks

Relationships with other risks

- Risk of Injury and Compromised Performance Due to EVA Operations
- Risk of Altered Sensorimotor/Vestibular Function Impacting Critical Mission Tasks
- Risk of Performance Decrement and Crew Illness Due to Inadequate Food and Nutrition
- Risk of Adverse Outcomes Due to Inadequate Human Systems Integration Architecture
- Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders
- Risk of Reduced Crew Health and Performance Due to Hypoxia [inactive]
- Risk of Bone Fracture due to Spaceflight-induced Changes to Bone

- Risk of Performance Decrements and Adverse Health Outcomes Resulting from Sleep Loss, Circadian Desynchronization, and Work Overload

4. KNOWLEDGE BASE

a. Gaps in knowledge

Link to the current gaps on the HRR

HRP Aerobic Gaps

- CV2: What is VO2max in-flight and immediately post-flight?
- A4: Establish VO2 standards for successful completion of mission tasks.
- A6: Develop pre-flight, in-flight, and post-flight evaluations to determine if VO2 standards are met.
- A7: Develop the most efficient and effective exercise program for the maintenance of VO2 standards.
- A9: Identify and validate exploration countermeasure hardware for the maintenance of VO2 standards.

HRP Muscle Strength and Endurance Gaps

- M2: Characterize in-flight and post-flight muscle performance.
- M4: Establish muscle function standards for successful completion of mission tasks.
- M6: Develop pre-flight, in-flight, and post-flight evaluations to determine if muscle function standards are met.
- M7: Develop the most efficient and effective exercise program for the maintenance of muscle function.
- M9: Identify and validate exploration countermeasure hardware for the maintenance of muscle function.
- M14: Identify adjuncts to exercise countermeasures that can be used to better mitigate muscle loss.
- M23: Determine if factors other than unloading contribute to muscle atrophy during space flight.
- M24: Characterize the time course of changes in muscle protein turnover, muscle mass, and function during long duration space flight.

Additional Gaps related to aerobic and muscle strength and endurance exercise

- EVA
  - EVA-101: Determine limitations of EVA performance and physiological metrics shortly post-landing on a planetary surface (with compromised physiology based on flight duration)
- Sensorimotor
  - SM-101: Characterize the effects of short and long-duration weightlessness, with and without deep-space radiation, on postural control and locomotion (gross motor control) after G transitions.
  - SM-104: Evaluate how weightlessness-induced changes in sensorimotor/vestibular function relate to and/or interact with changes in other brain functions (sleep, cognition, attention).
- Performance decrement due to inadequate nutrition
  - FN-101: Determine the nutritional requirements that would support optimal physiological and psychological performance and prevent disease during different

phases of an exploration mission: outbound weightless journey, in-flight EVA, surface operations and return weightless journey, during and after the mission.
    - FN-402: Develop strategies for use of pre-packaged food and in-flight crops as a physiological countermeasure (for aspects such as radiation/oxidative stress, cardiovascular health, bone and renal stone, SANS, exercise, immune, MicroHost, mood, cognition, performance, sleep) and determine the human health risk/benefit to food system resource trade of promising strategies.
- Inadequate Human Systems Integration
    - HSIA-101: We need to identify the Human Systems Integration (HSI) – relevant crew health and performance outcomes, measures, and metrics, needed to characterize and mitigate risk, for future exploration missions.
    - HSIA-201: We need to evaluate the demands of future exploration habitat/vehicle systems and mission scenarios (e.g. increased automation, multi-modal communication) on individuals and teams, and determine the risks these demands pose to crew health and performance.
- Adverse Cognitive or Behavioral Conditions
    - BMed-105: Given the potentially negative spaceflight associated CNS/cognitive changes and behavioral experiences of stressors during long-duration missions (e.g., isolation, confinement, reduced sensory stimulation, altered gravity, space radiation), what are validated medical or dietary countermeasures to mitigate stressors impacting on CNS / cognition / behavioral health?
    - BMed-108: Given each crewmember will experience multiple spaceflight hazards simultaneously, we need to identify and characterize the potential additive, antagonistic, or synergistic impacts of multiple stressors (e.g., space radiation, altered gravity, isolation, altered immune, altered sleep) on crew health and/or CNS/ cognitive functioning to develop threshold limits and validate countermeasures for any identified adverse crew health and/or operationally-relevant performance outcomes.
- Space flight-induced changes to bone
    - Osteo 4: We don't know the contribution of each risk factor on bone loss and recovery of bone strength, and which factors are the best targets for countermeasure application.
    - Osteo 7: We need to identify options for mitigating early onset osteoporosis before, during and after spaceflight.
- Performance decrements due to sleep loss
    - Sleep-101: Given each crew member will experience multiple spaceflight hazards simultaneously, we need to identify and characterize the potential additive, antagonistic, or synergistic impacts of multiple stressors (e.g., Space Radiation, Altered Gravity, Isolation, altered immune, altered sleep) on crew sleep-wake cycles and/or circadian shifting, health and/or CNS/cognitive functioning to identify any identified adverse individual or team crew health, and/or operationally-relevant performance outcomes.

b. State of knowledge/future work

Current state of knowledge on Aerobic Capacity from ISS

- Aerobic capacity is reduced on average about 10% pre to postflight with ISS countermeasures provided to crew

- There is large individual variability of ISS crew for aerobic capacity changes
    - About 30% of crew maintain or improve aerobic capacity
    - About 70% of crew show reduced aerobic capacity
    - About 53% of crew show reduced aerobic capacity greater than 10% loss
    - About 15% of crew show reduced aerobic capacity greater than 20% loss

Current state of knowledge on Muscle Strength and Endurance from ISS

- Isokinetic knee strength is reduced on average about 15% from pre to postflight with ISS countermeasures provided to crew
- There is large individual variability of ISS crew for isokinetic knee extension strength changes
    - About 23% of crew maintain or improve knee strength
    - About 77% of crew show reduced knee strength
    - About 63% of crew lose greater than 10% knee muscle strength
    - About 31% of crew lose greater than 20% knee muscle strength

- Isokinetic knee endurance is reduced on average about 17%% from pre to postflight when ISS countermeasures are provided to crew
- There is large individual variability of ISS crew for isokinetic knee extension endurance changes
    - About 12% of crew maintain or improve knee endurance
    - About 88% of crew show reduced knee endurance
    - About 55% of crew lose greater than 10% knee muscle endurance
    - About 22% of crew lose greater than 20% knee muscle endurance

Despite four decades of effort, success in prevention of space flight muscle atrophy and skeletal muscle functional deficits has not yet been achieved in every case, although progress has been made. Gaps in knowledge have prevented us from implementing a countermeasures program that will fully mitigate the risks of losing muscle mass, function, and endurance during exposure to the microgravity of space flight, particularly during long-duration missions. There are also gaps in knowledge about working and living in partial-g environments and the effect that wearing an EVA suit has on human performance in such an environment. The countermeasure readiness level for exercise is very high and developing countermeasures for humans is the highest priority.

The major knowledge gaps that must be addressed by future research to mitigate this risk of loss of aerobic capacity, skeletal muscle mass, function, and endurance include, but are not limited to, the following:

Exercise prescriptions as a countermeasure

- The minimal dose of exercise needed to maintain aerobic capacity and muscle strength and endurance is unknown. This will require a detailed characterization of the exercise completed (i.e., frequency, intensity, time, volume, progression) in-flight and how these affect pre to postflight aerobic and strength changes.
- It is unknown why large variability of exercise response occurs. A standardized, individualized exercise prescription is needed to minimize the exercise response variability.
- Standards of exercise prescriptions need to be developed that normalize aerobic capacity and muscle strength and endurance to preflight measurements of maximal

capacity or body size. The dose of exercise as it relates to maximal capabilities (%, VO_2peak, %1RM, or % body mass) can guide appropriate prescription needed to maintain fitness.

- Real time monitoring/feedback of exercise completed for adjustments in the prescription of exercise based on changes of in-flight aerobic capacity and muscle strength and endurance measures will need careful evaluation for in-flight individualized exercise prescription.
- Currently, no standard in-flight measurement of strength exists.
    - Importance of characterizing in-flight strength (IMTP)
- Need to understand how aerobic capacity and muscle strength and endurance relate to functional fitness.
- Exercise genetics may improve our understanding of responders and non-responders and may provide improvements in exercise prescriptions.
- Preflight fitness
    - Exploration vehicle exercise hardware will be limited in capabilities. A strategy may be implemented to provide crew with extensive preflight training to improve aerobic capacity and muscle strength and endurance to improve their aerobic capacity and strength reserves.
- Female astronaut and age
    - Individualized exercise prescription will need to further address differences in sex and age. While early missions (Mercury-Apollo) were exclusively male; subsequent and future crews consist of mixed gender crews with increasing heterogeneity. There has also been a gradual increase in operational age of the astronaut population (from age 34±4 years in the 1960s to 45±4 in the 2010s [543]. The expectation that this trend will continue in the future is unclear, and the current age range appears to narrow as astronaut retirement age has decreased over the past few years. Nevertheless, astronaut heterogeneity needs to be considered for countermeasure optimization.
- It is important to understand how interruptions in the exercise program can be handled during long-duration exploration programs. Is it acceptable to periodize exercise to provide planned rest periods? How long could crewmembers go without resistance exercise? What adjunct therapies could be provided in the event exercise must be discontinued?
- Rehabilitation techniques in micro- and partial-g are completely unknown. It is unknown whether chronically unloaded muscle is more vulnerable to musculoskeletal injury (including cartilage and joint) upon reloading in a partial-g environment.
- Understanding the time course of loss of aerobic capacity, muscle mass, strength and function is important in order to titrate the exercise prescription with the hardware needs for missions of differing durations.

Exercise and drug /nutrition countermeasures

- Exercise and drug/supplement combinations as synergistic countermeasures are unknown.
- It is important to understand whether the dominant mechanism underlying the space flight atrophic process is protein degradation via the ubiquiting-proteasome axis or decreased protein synthesis due to loss of ribosomal RNA and pre-mRNA of actin and myosin. This knowledge can inform the choice of pharmaceutical or nutritional supplements as adjuncts to exercise.
- There is a potential for pharmaceutical interventions based on the anabolic effectiveness of compounds, such as testosterone. Bed rest studies have

demonstrated that adjuvant treatment with testosterone (a naturally occurring anabolic hormone) effectively protects against declines in lean body mass in males [464, 544]. However, the inclusion of an exercise countermeasure in addition to low-dose testosterone treatment was necessary to protect against decreases in muscle [7, 465]. More research is needed to determine whether comparable findings are seen in females and with varied exercise prescriptions.

Exploration vehicle exercise hardware

- Exploration vehicles for long-duration space flight will not have the same ISS capabilities. Therefore, with assumed less exercise hardware capabilities, exercise prescriptions may need further adjustments in frequency, intensity, time, volume and/or progression.
- Exploration vehicle hardware countermeasures will need careful evaluations on their acute and chronic exercise responses that include metabolic rate, oxygen uptake, heart rate, work relationships, axial spinal loading, behavior health, and force plate load profiles. Moreover, exercise responses on the hardware need careful evaluation on the chronic adaptations and differences in 1 g compared to 0 g.

Exercise and other risks

- Need to understand how exercise effects other risks (i.e., bone, sensorimotor, behavior health, nutrition, EVA operations).
- Exercise, while necessary, is likely the single biggest challenge to vehicle Environmental Control and Life Support Systems (ECLSS) sizing as well as food and water supply. Defining minimum requirements to maintain crew health must be counterbalanced with the reality that even if crew time (the current limitation) was available, exercise capacity will likely still be constrained by ECLSS and food systems.
- Unloading induced insulin resistance has been commonly reported in the research literature [545-547], though it has generally not been observed to cause major health problems on 6-month missions. It remains to be determined whether longer duration missions lead to clinically relevant insulin resistance of skeletal muscle [545].

Exercise requirements and standards

- Need to characterize aerobic and strength functional requirements
    - Lunar missions
    - Interplanetary travel to mars
    - Partial g EVA standards
    - 0g EVA and Vehicle egress
- The following standards (NASA STD-3001 VOLUME 1) need careful review and updates need to be specific to microgravity, Lunar, and Martian environments
    - 4.1.1 Microgravity EVA aerobic capacity standard
    - 4.1.2 Celestial Surface EVA aerobic capacity standard
    - 4.1.3 In-mission aerobic capacity standard
    - 4.6.1 Pre-Mission muscle strength and Function standards
    - 4.5.2 In mission skeletal muscle strength
- Fitness for duty: Crew health and fitness standards for operational performance have been evaluated and implemented for microgravity environments; however, there are no such standards for partial gravity environments. Ongoing work in the Human Physiology, Protection, Performance, and Operations Laboratory aims to develop and evaluate Aerobic and Strength thresholds for partial gravity EVAs utilizing ground-based simulations to establish a potential model of partial gravity EVA performance

specific to 1/6 g. In order to achieve these objectives, work is underway evaluating the occupational health and physiology literature, mining data from historical partial-gravity EVAs (i.e., Apollo missions), and leveraging data from ongoing characterizations of the physiological demands during simulated partial gravity EVAs.

i) If closed or phase-out: State of evidence at close-out mitigation, remaining gaps, ramifications to risk posture if mitigation is not adopted.

5. CONCLUSIONS

Aerobic capacity as well as muscle strength and endurance decrease following short and long-duration space flight and after bed rest. The reduced SV, perhaps secondary to lower plasma volume and decreased diastolic filling, is believed to be a major influence on exercise capacity, especially during orthostatic stress. Loss of strength is also associated with muscle atrophy and change in fiber type. Both the loss in aerobic capacity and muscle strength are dependent on the duration of space flight and the exercise countermeasures crew perform in-flight.

Although no controlled studies of exercise countermeasure effectiveness have been conducted during space flight, data from bed rest studies have demonstrated that countermeasures provide protection against losses in aerobic capacity and muscle strength and endurance. Recent long-duration in-flight studies on the ISS have reported that higher intensity exercise and reduced exercise time are comparable to the traditional exercise prescriptions. However, the review of recent preliminary data from ISS missions finds high interindividual variability in the response to the exercise prescriptions. The dose of exercise completed is a major gap in knowledge important for long-duration missions to the Lunar and Martian surface.

Although there are no reports of impaired performance of activities in microgravity, decreased aerobic capacity and muscle strength and endurance may affect the efficiency of work and the intensity and duration for which the work can be performed for EVAs in microgravity and partial gravity environments. Crewmembers should also maintain a level of fitness that provides completing the energy expenditures (%VO_2peak) needed for EVAs taking place in microgravity or partial gravity operational exploration scenarios. Additionally, crew must have and maintain their exercise capacity reserves to react to emergency scenarios. Unfortunately, the required minimum level of fitness cannot be fully determined until mission scenarios, critical mission tasks, and suit design and Notional Design Reference EVA operations are defined. Furthermore, the standards of requirements for aerobic and muscle strength and endurance will need careful review and will need to be updated to meet specific microgravity, Lunar, and Martian environments.

It will be important for all crew to maintain fitness for exploration missions. For future Artemis missions to the Lunar surface, exploration vehicles will not have the similar hardware capabilities as on the ISS due to the limited volume. This may alter the effectiveness of hardware to provide adequate stress on the body allowing for maintenance of strength and bone density. Newer, more compact, exploration exercise hardware is currently being evaluated (E4D and Orion Flywheel). The E4D is currently in development and has not been evaluated for exercise responses. Metabolic rate, oxygen uptake, heart rate, work relationships, and force plate load profiles are needed to assess the stress of the hardware on the human body. Moreover, exercise responses on the hardware need careful evaluation on the chronic adaptations. Lastly, in-flight evaluation of hardware exercise response may differ in 0 g compared to 1 g. Consequently, it cannot be assumed that the stress on the body will be the same in both environments. Understanding this has a direct impact on exercise prescriptions and crew health risk mitigation.

This report has reviewed evidence from human and animal space flight and ground-based analogs as they impact the decrements in aerobic fitness, skeletal muscle mass, strength, and endurance, and the relevant findings have been presented. Most importantly, new data from a large dataset of MEDB data on long-duration ISS missions has been added, providing clear insight into the significant variability of exercise response of crew and demonstrating that groups of crew that return to Earth with greater than 10% and 20% loss. Data from human space flight and ground-based studies are narrowing in on the required exercise paradigms but thus far still provide an incomplete answer to an effective approach for maintaining skeletal muscle function of all human space travelers.

6. LIST OF TABLES AND FIGURES

7. RESOURCES

A. Prior evidence report

B. Index service entries for peer-reviewed papers study protocols (PubMed et al.)

C. NTRS/STI

D. Task Book (Internal repository of study protocols for HRP-funded intra- and extra-mural research is there a publicly viewable equivalent? Should there be?)

E. HRR risk page

F. HSRB DAG

G. LSDA/GeneLab

H. HRP CMR (Computational Model Repository)

I. Publications

8. REFERENCES

1. Lee, S.M., et al., *Inflight exercise affects stand test responses after space flight.* Med Sci Sports Exerc, 1999. **31**(12): p. 1755-62.
2. Watenpaugh, D.E., et al., *Supine lower body negative pressure exercise during bed rest maintains upright exercise capacity.* J Appl Physiol (1985), 2000. **89**(1): p. 218-27.
3. Schneider, S.M., et al., *WISE-2005: exercise and nutrition countermeasures for upright VO2pk during bed rest.* Med Sci Sports Exerc, 2009. **41**(12): p. 2165-76.
4. Lee, S.M., et al., *Supine LBNP exercise maintains exercise capacity in male twins during 30-d bed rest.* Med Sci Sports Exerc, 2007. **39**(8): p. 1315-26.
5. LeBlanc, A., et al., *Regional muscle loss after short duration spaceflight.* Aviat Space Environ Med, 1995. **66**(12): p. 1151-4.
6. LeBlanc, A.D., et al., *Regional changes in muscle mass following 17 weeks of bed rest.* J Appl Physiol (1985), 1992. **73**(5): p. 2172-8.
7. Ploutz-Snyder, L.L., et al., *Exercise Training Mitigates Multisystem Deconditioning during Bed Rest.* Med Sci Sports Exerc, 2018. **50**(9): p. 1920-1928.
8. Lee, S.M., W.J. Williams, and S.M. Schneider, *Role of skin blood flow and sweating rate in exercise thermoregulation after bed rest.* J Appl Physiol (1985), 2002. **92**(5): p. 2026-34.
9. Trappe, T., et al., *Cardiorespiratory responses to physical work during and following 17 days of bed rest and spaceflight.* J Appl Physiol, 2006. **100**(3): p. 951-7.
10. Hargens, A.R. and D.E. Watenpaugh, *Cardiovascular adaptation to spaceflight.* Med Sci Sports Exerc, 1996. **28**(8): p. 977-82.
11. Hargens, A.R. and L. Vico, *Long-duration bed rest as an analog to microgravity.* J Appl Physiol (1985), 2016. **120**(8): p. 891-903.
12. Trudel, G., et al., *Hemolysis contributes to anemia during long-duration space flight.* Nat Med, 2022. **28**(1): p. 59-62.

13. Berry, C.A. and A.D. Catterson, *Pre-Gemini Medical Predictions Versus Gemini Flight Results, NASA SP-138*, in *Gemini Sumary Conference, NASA SP-138*. 1967, National Aeronautics and Space Administration.
14. Greenisen, M.C., Hayes, J., Siconolfi, S. Moore, A., *Functional Performance Evaluation*, in *Extended Duration Orbiter Medical Project Final Report 1989-1995*, C.F. Sawin, Taylor, G.R., Smith, W.L., Editor. 1999. p. 3.1-3.24.
15. Dietlein, L.F. and R.M. Rapp, *Experiment M-3: Inflight exercise work tolerance*, in *Gemini Midprogram Conference Including Experimental Results, NASA SP-121*. 1966, National Aeronautics and Space Administration.
16. Alkner, B.A. and P.A. Tesch, *Knee extensor and plantar flexor muscle size and function following 90 days of bed rest with or without resistance exercise.* Eur J Appl Physiol, 2004. **93**(3): p. 294-305.
17. Bamman, M.M., et al., *Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution.* J Appl Physiol (1985), 1998. **84**(1): p. 157-63.
18. Berry, P., I. Berry, and C. Manelfe, *Magnetic resonance imaging evaluation of lower limb muscles during bed rest--a microgravity simulation model.* Aviat Space Environ Med, 1993. **64**(3 Pt 1): p. 212-8.
19. Dudley, G.A., et al., *Changes of muscle function and size with bedrest.* Physiologist, 1989. **32**(1 Suppl): p. S65-6.
20. Ferrando, A.A., et al., *Prolonged bed rest decreases skeletal muscle and whole body protein synthesis.* Am J Physiol, 1996. **270**(4 Pt 1): p. E627-33.
21. Gogia, P., et al., *Bed rest effect on extremity muscle torque in healthy men.* Arch Phys Med Rehabil, 1988. **69**(12): p. 1030-2.
22. Kawakami, Y., et al., *Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise.* Eur J Appl Physiol, 2001. **84**(1-2): p. 7-12.
23. Kortebein, P., et al., *Effect of 10 days of bed rest on skeletal muscle in healthy older adults.* JAMA, 2007. **297**(16): p. 1772-4.
24. LeBlanc, A., et al., *Calf muscle area and strength changes after five weeks of horizontal bed rest.* Am J Sports Med, 1988. **16**(6): p. 624-9.
25. Mulder, E.R., et al., *Influence of vibration resistance training on knee extensor and plantar flexor size, strength, and contractile speed characteristics after 60 days of bed rest.* J Appl Physiol (1985), 2009. **107**(6): p. 1789-98.
26. Paddon-Jones, D., et al., *Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest.* J Clin Endocrinol Metab, 2004. **89**(9): p. 4351-8.
27. Suzuki, Y., et al., *Decrease of bone mineral density and muscle and/or strength in the leg during 20 days horizontal bed rest.* J Gravit Physiol, 1996. **3**(2): p. 42-3.
28. Trappe, T.A., et al., *Influence of concurrent exercise or nutrition countermeasures on thigh and calf muscle size and function during 60 days of bed rest in women.* Acta Physiol (Oxf), 2007. **191**(2): p. 147-59.
29. Administration, N.A.a.S., *Apollo Program Summary Report.* 1975.
30. *NASA-STD-3001, REVISION B NASA SPACE FLIGHT HUMAN-SYSTEM STANDARD: VOLUME 1: CREW HEALTH* 2022.
31. Lee, S., et al., *Supine lower body negative pressure treadmill exercise maintains upright exercise capacity in women and men after 30-d bed rest.* Med Sci Sports Exerc, 2005. **37**(5 Suppl): p. S36.
32. Pescatello, L.S. and American College of Sports Medicine., *ACSM's guidelines for exercise testing and prescription*. 9th ed. 2014, Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health. xxiv, 456 p.

33. Thornton, W.E., Hoffler, G.W., Rummel, J.A., *Anthropomorphic changes and fluid shifts*, in *Biomedical Results of Skylab*, R.S. Johnston, Dietlein, L.F., Editor. 1977, NASA: Washington, D.C. p. 330-338.
34. Thornton, W.E., Rummel, J.A., *Muscular deconditioning and its prevention in spaceflight.*, in *Biomedical Results from Skylab*, R.S. Johnston, Dietlein, L.F., Editor. 1977, NASA: Washington, D.C. p. 191-197.
35. Fortney, S.M., et al., *Body temperature and thermoregulation during submaximal exercise after 115-day spaceflight.* Aviat Space Environ Med, 1998. **69**(2): p. 137-41.
36. Waligora JM., H.W., Humbert GF, Nelson LJ, Vogel SJ, Kuznetz LH, *Apollo Experience Report, Assessment of Metabolic Expenditures.* 1975.
37. Convertino, V.A., *Cardiovascular consequences of bed rest: effect on maximal oxygen uptake.* Med Sci Sports Exerc, 1997. **29**(2): p. 191-6.
38. Katayama, K., et al., *Acceleration with exercise during head-down bed rest preserves upright exercise responses.* Aviat Space Environ Med, 2004. **75**(12): p. 1029-35.
39. *EVA Exploration Workshop, Exploration EVA System Concept of Operations Summary for Artemis Phase 1 Lunar Surface Mission* 2020.
40. Fleg, J.L., et al., *Accelerated longitudinal decline of aerobic capacity in healthy older adults.* Circulation, 2005. **112**(5): p. 674-82.
41. Ried-Larsen, M., H.M. Aarts, and M.J. Joyner, *Effects of strict prolonged bed rest on cardiorespiratory fitness: systematic review and meta-analysis.* J Appl Physiol (1985), 2017. **123**(4): p. 790-799.
42. Levine, B.D., et al., *Maximal exercise performance after adaptation to microgravity.* J Appl Physiol (1985), 1996. **81**(2): p. 686-94.
43. Harbo, T., J. Brincks, and H. Andersen, *Maximal isokinetic and isometric muscle strength of major muscle groups related to age, body mass, height, and sex in 178 healthy subjects.* Eur J Appl Physiol, 2012. **112**(1): p. 267-75.
44. Lackner, J.R. and P. Dizio, *Space motion sickness.* Exp Brain Res, 2006. **175**(3): p. 377-99.
45. Link, M.M., *Space Medicine in Project Mercury.* 1965.
46. Boda, W.L., et al., *Supine lower body negative pressure exercise simulates metabolic and kinetic features of upright exercise.* J Appl Physiol (1985), 2000. **89**(2): p. 649-54.
47. Moore, A.D., Jr., et al., *Peak exercise oxygen uptake during and following long-duration spaceflight.* J Appl Physiol (1985), 2014. **117**(3): p. 231-8.
48. Huang, T.W. and A.D. Kuo, *Mechanics and energetics of load carriage during human walking.* J Exp Biol, 2014. **217**(Pt 4): p. 605-13.
49. Paloski W., O.C., Bloomberg JJ, Resche MF, Wood SJ, Harm DL, Peters BT, Mulavara AP, Locke JP, Stone LS, *Risk of sensory-motor performance failures affecting vehicle control during space missions: A review of the evidence.* Journal of Gravitational Physiology, 2008. **15**(2).
50. Cramer, M.N. and O. Jay, *Biophysical aspects of human thermoregulation during heat stress.* Auton Neurosci, 2016. **196**: p. 3-13.
51. Scott, J.M., et al., *Teleguided self-ultrasound scanning for longitudinal monitoring of muscle mass during spaceflight.* iScience, 2021. **24**(4): p. 102344.
52. English, K.L., et al., *High intensity training during spaceflight: results from the NASA Sprint Study.* NPJ Microgravity, 2020. **6**: p. 21.
53. Stavnichuk, M., et al., *A systematic review and meta-analysis of bone loss in space travelers.* NPJ Microgravity, 2020. **6**: p. 13.
54. Vico, L. and A. Hargens, *Skeletal changes during and after spaceflight.* Nat Rev Rheumatol, 2018. **14**(4): p. 229-245.
55. Leblanc, A., et al., *Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight.* Osteoporos Int, 2013. **24**(7): p. 2105-14.

56. Grimm, D., et al., *The impact of microgravity on bone in humans.* Bone, 2016. **87**: p. 44-56.
57. Gopalakrishnan, R., et al., *Muscle volume, strength, endurance, and exercise loads during 6-month missions in space.* Aviat Space Environ Med, 2010. **81**(2): p. 91-102.
58. English, K.L., et al., *Leucine partially protects muscle mass and function during bed rest in middle-aged adults.* Am J Clin Nutr, 2016. **103**(2): p. 465-73.
59. English, K.L., et al., *Isokinetic Strength Changes Following Long-Duration Spaceflight on the ISS.* Aerosp Med Hum Perform, 2015. **86**(12 Suppl): p. A68-A77.
60. Morita, H., C. Abe, and K. Tanaka, *Long-term exposure to microgravity impairs vestibulo-cardiovascular reflex.* Sci Rep, 2016. **6**: p. 33405.
61. Gallo, C., L. Ridolfi, and S. Scarsoglio, *Cardiovascular deconditioning during long-term spaceflight through multiscale modeling.* NPJ Microgravity, 2020. **6**: p. 27.
62. Prisk, G.K., *Microgravity and the lung.* J Appl Physiol (1985), 2000. **89**(1): p. 385-96.
63. Fitts, R.H., D.R. Riley, and J.J. Widrick, *Physiology of a microgravity environment invited review: microgravity and skeletal muscle.* J Appl Physiol (1985), 2000. **89**(2): p. 823-39.
64. Sieck, G.C., *Highlighted topics series: physiology of a microgravity environment.* J Appl Physiol (1985), 2000. **89**(1): p. 1-2.
65. Hargens, A.R., R. Bhattacharya, and S.M. Schneider, *Space physiology VI: exercise, artificial gravity, and countermeasure development for prolonged space flight.* Eur J Appl Physiol, 2013. **113**(9): p. 2183-92.
66. Ade, C.J., R.M. Broxterman, and T.J. Barstow, *VO(2max) and Microgravity Exposure: Convective versus Diffusive O(2) Transport.* Med Sci Sports Exerc, 2015. **47**(7): p. 1351-61.
67. Komorowski, M., S. Fleming, and A.W. Kirkpatrick, *Fundamentals of Anesthesiology for Spaceflight.* J Cardiothorac Vasc Anesth, 2016. **30**(3): p. 781-90.
68. Kozlovskaya, I.B. and A.I. Grigoriev, *Russian system of countermeasures on board of the International Space Station (ISS): the first results.* Acta Astronaut, 2004. **55**(3-9): p. 233-7.
69. Smith, S.M., et al., *Evaluation of treadmill exercise in a lower body negative pressure chamber as a countermeasure for weightlessness-induced bone loss: a bed rest study with identical twins.* J Bone Miner Res, 2003. **18**(12): p. 2223-30.
70. Bishop, P.A., et al., *Carbon dioxide accumulation, walking performance, and metabolic cost in the NASA launch and entry suit.* Aviat Space Environ Med, 1999. **70**(7): p. 656-65.
71. Portree, D.S. and R.C. Trevino, *Walking to Olympus: An EVA Chronology*. 1997, NASA History Office: Washington D.C.
72. White, S.C. and C.A. Berry, *Postlaunch Memorandum Report for Mercury-Atlas No. 7 (MA-7)*. Postlaunch Memorandum Report for Mercury-Atlas No. 7 (MA-7), ed. E.M. Fields, et al. 1962, Manned Spacecraft Center, Cape Canaveral, Florida: National Aeronautics and Space Administration. 7-27 - 7-61.
73. Berry, C.A., et al., *Results of the Third United States Manned Orbital Space Flight, NASA SP-12*. Chapter 3, Aeromedical Analysis. 1962, Washington, D.C.: National Aeronautics and Space Administration. 23-36.
74. Johnston, R.S., Dietlein, JF *Biomedical Results from Skylab. Muscular deconditioning and its prevention in space flight.* 1977.
75. Berry, C.A., *Summary of medical experience in the Apollo 7 through 11 manned spaceflights.* Aerosp Med, 1970. **41**(5): p. 500-19.
76. Rummel, J.A., E.L. Michel, and C.A. Berry, *Physiological response to exercise after space flight--Apollo 7 to Apollo 11.* Aerosp Med, 1973. **44**(3): p. 235-8.

77. Rummel, J.A., Sawin, C.F., Michel, E.L. and M.S. E.L. Michel, *Exercise Response*, in *Biomedical Results of Apollo*, R.S. Johnston, Dietlein, L.F., Berry, C.A., Editor. 1975, NASA: Washington, D.C.
78. Rummell, J.A., et al., *Physiological response to exercise after space flight--Apollo 14 through Apollo 17.* Aviat Space Environ Med, 1975. **46**(5): p. 679-83.
79. Maxfield, M.E. and L. Brouha, *Validity of Heart Rate as an Indicator of Cardiac Strain.* J Appl Physiol, 1963. **18**: p. 1099-104.
80. Kim, T.S., H. Rahn, and L.E. Farhi, *Estimation of true venous and arterial PCO2 by gas analysis of a single breath.* J Appl Physiol, 1966. **21**(4): p. 1338-44.
81. Buderer, M.C., et al., *Use of the single-breath method of estimaring cardiac output during exercise-stress testing.* Aerosp Med, 1973. **44**(7): p. 756-63.
82. Leach, C.S., W.C. Alexander, and P.C. Johnson, *Endocrine, Electrolyte and Fluid Volume Changes Association With Apollo Missions*, in *Biomedical Results of Apollo, NASA SP-368*, R.S. Johnston, L.F. Dietlein, and C.A. Berry, Editors. 1975, Scientific and Technical Information Office, NASA: Washington, D.C. p. 163-184.
83. Dietlein, L.F., *Summary and conclusions.*, in *Biomedical Results of Apollo*, R.S. Johnston, Dietlein, L.F., Berry, C.A., Editor. 1975, NASA: Washington, D.C.
84. Michel, E.L., J.A. Rummel, and C.F. Sawin, *Skylab experiment M-171 "Metabolic Activity"--results of the first manned mission.* Acta Astronaut, 1975. **2**(3-4): p. 351-65.
85. Michel, E.L., et al., *Results of Skylab medical experiment 171: Metabolic activity*, in *Biomedical Results from Skylab*, R.S. Johnson and L.F. Dietlein, Editors. 1977, National Aeronautics and Space Administration: Washington, DC. p. 372-387.
86. Buderer, M.C., et al., *Exercise cardiac output following Skylab missions: the second manned Skylab mission.* Aviat Space Environ Med, 1976. **47**(4): p. 365-72.
87. Johnson, P.C., T.B. Driscoll, and A.D. LeBlanc, *Blood Volume Changes*, in *Biomedical Results from Skylab*, R.S. Johnson and L.F. Dietlein, Editors. 1977, National Aeronautics and Space Administration: United States. p. 235-241.
88. Sawin, C.F., J.A. Rummel, and E.L. Michel, *Instrumented personal exercise during long-duration space flights.* Aviat Space Environ Med, 1975. **46**(4 Sec 1): p. 394-400.
89. Whedon, G.D., Lutwak, L., Rambaut, P.C., Whittle, M.W.,Smith, M.C., Reid, J., Leach, C., Stadler, C.J., Sanford, D.D., *Mineral and nitrogen metabolic studies --Experiment M071*, in *Biomedical Results of Skylab*, R.S. Johnston, Dietlein, L.F., Editor. 1977, NASA: Washington, D.C. p. 164-174.
90. Stein, T.P., M.J. Leskiw, and M.D. Schluter, *Diet and nitrogen metabolism during spaceflight on the shuttle.* J Appl Physiol (1985), 1996. **81**(1): p. 82-97.
91. Shykoff, B.E., et al., *Cardiovascular response to submaximal exercise in sustained microgravity.* J Appl Physiol (1985), 1996. **81**(1): p. 26-32.
92. Alfrey, C.P., et al., *Control of red blood cell mass in spaceflight.* J Appl Physiol (1985), 1996. **81**(1): p. 98-104.
93. Moore, A.D.J., et al., *Maximal exercise as a countermeasure to orthostatic intolerance after spaceflight.* Med Sci Sports Exerc, 2001. **33**(1): p. 75-80.
94. Convertino, V.A., *Potential benefits of maximal exercise just prior to return from weightlessness.* Aviat Space Environ Med, 1987. **58**(6): p. 568-72.
95. Trappe, T., et al., *Cardiorespiratory responses to physical work during and following 17 days of bed rest and spaceflight.* J Appl Physiol (1985), 2006. **100**(3): p. 951-7.
96. Edgerton, V.R., et al., *Human fiber size and enzymatic properties after 5 and 11 days of spaceflight.* J Appl Physiol (1985), 1995. **78**(5): p. 1733-9.
97. Zhou, M.Y., et al., *Myosin heavy chain isoforms of human muscle after short-term spaceflight.* J Appl Physiol (1985), 1995. **78**(5): p. 1740-4.
98. Itai, Y., Y. Kariya, and Y. Hoshino, *Morphological changes in rat hindlimb muscle fibres during recovery from disuse atrophy.* Acta Physiol Scand, 2004. **181**(2): p. 217-24.

99. Jaspers, S.R. and M.E. Tischler, *Atrophy and growth failure of rat hindlimb muscles in tail-cast suspension.* J Appl Physiol Respir Environ Exerc Physiol, 1984. **57**(5): p. 1472-9.
100. Steffen, J.M., et al., *Age effects on rat hindlimb muscle atrophy during suspension unloading.* J Appl Physiol (1985), 1990. **68**(3): p. 927-31.
101. LeBlanc, A., et al., *Muscle volume, MRI relaxation times (T2), and body composition after spaceflight.* J Appl Physiol (1985), 2000. **89**(6): p. 2158-64.
102. Tesch, P.A., et al., *Effects of 17-day spaceflight on knee extensor muscle function and size.* Eur J Appl Physiol, 2005. **93**(4): p. 463-8.
103. Trappe, S.W., et al., *Comparison of a space shuttle flight (STS-78) and bed rest on human muscle function.* J Appl Physiol, 2001. **91**(1): p. 57-64.
104. Trappe, S., *Effects of spaceflight, simulated spaceflight and countermeasures on single muscle fiber physiology.* J Gravit Physiol, 2002. **9**(1): p. P323-6.
105. Narici, M., et al., *Effects of 17-day spaceflight on electrically evoked torque and cross-sectional area of the human triceps surae.* Eur J Appl Physiol, 2003. **90**(3-4): p. 275-82.
106. Riley, D.A., et al., *Decreased thin filament density and length in human atrophic soleus muscle fibers after spaceflight.* J Appl Physiol (1985), 2000. **88**(2): p. 567-72.
107. Riley, D.A., et al., *Thin filament diversity and physiological properties of fast and slow fiber types in astronaut leg muscles.* J Appl Physiol, 2002. **92**(2): p. 817-25.
108. Widrick, J.J., et al., *Effect of a 17 day spaceflight on contractile properties of human soleus muscle fibres.* J Physiol, 1999. **516 (Pt 3)**: p. 915-30.
109. Widrick, J.J., et al., *Functional properties of slow and fast gastrocnemius muscle fibers after a 17-day spaceflight.* J Appl Physiol, 2001. **90**(6): p. 2203-11.
110. Edgerton, V.R., et al., *Human fiber size and enzymatic properties after 5 and 11 days of spaceflight.* J Appl Physiol, 1995. **78**(5): p. 1733-9.
111. Trappe, S.W., et al., *Comparison of a space shuttle flight (STS-78) and bed rest on human muscle function.* J Appl Physiol (1985), 2001. **91**(1): p. 57-64.
112. Siconolfi, S.F., et al., *Comparing the effects of two in-flight aerobic exercise protocols on standing heart rates and VO(2peak) before and after space flight.* J Clin Pharmacol, 1994. **34**(6): p. 590-5.
113. Greenisen, M.C., et al., *Functional performance evaluation*, in *Extended Duration Orbiter Medical Project, Final Report 1989-1995, NASA/SP-1999-534*, S.F. Sawin, G.R. Taylor, and W.L. Wanda, Editors. 1999, National Aeronautics And Space Administration: Houston, TX. p. 3.1-3.24.
114. LeBlanc, A., et al., *Muscle volume, MRI relaxation times (T2), and body composition after spaceflight.* J Appl Physiol, 2000. **89**(6): p. 2158-64.
115. Alexander, A.M., et al., *Prediction of Emergency Capsule Egress Performance.* Aerosp Med Hum Perform, 2019. **90**(9): p. 782-787.
116. Clemensen, P., et al., *A modified photo- and magnetoacoustic multigas analyzer applied in gas exchange measurements.* J Appl Physiol (1985), 1994. **76**(6): p. 2832-9.
117. Williams, D., et al., *Acclimation during space flight: effects on human physiology.* CMAJ, 2009. **180**(13): p. 1317-23.
118. Moore, A.D., Jr., et al., *Maximal exercise as a countermeasure to orthostatic intolerance after spaceflight.* Med Sci Sports Exerc, 2001. **33**(1): p. 75-80.
119. Ross, R., et al., *Precision exercise medicine: understanding exercise response variability.* Br J Sports Med, 2019. **53**(18): p. 1141-1153.
120. Bonafiglia, J.T., et al., *Exploring Differences in Cardiorespiratory Fitness Response Rates Across Varying Doses of Exercise Training: A Retrospective Analysis of Eight Randomized Controlled Trials.* Sports Med, 2021. **51**(8): p. 1785-1797.

121. Bonafiglia, J.T., N. Preobrazenski, and B.J. Gurd, *A Systematic Review Examining the Approaches Used to Estimate Interindividual Differences in Trainability and Classify Individual Responses to Exercise Training.* Front Physiol, 2021. **12**: p. 665044.
122. Meyler, S., L. Bottoms, and D. Muniz-Pumares, *Biological and methodological factors affecting V O 2 max response variability to endurance training and the influence of exercise intensity prescription.* Exp Physiol, 2021. **106**(7): p. 1410-1424.
123. Ross, R., L. de Lannoy, and P.J. Stotz, *Separate Effects of Intensity and Amount of Exercise on Interindividual Cardiorespiratory Fitness Response.* Mayo Clin Proc, 2015. **90**(11): p. 1506-14.
124. Macfarlane, D.J. and H.L. Wu, *Inter-unit variability in two ParvoMedics TrueOne 2400 automated metabolic gas analysis systems.* Eur J Appl Physiol, 2013. **113**(3): p. 753-62.
125. Fitts, R.H., et al., *Effects of prolonged space flight on human skeletal muscle enzyme and substrate profiles.* J Appl Physiol (1985), 2013. **115**(5): p. 667-79.
126. Fitts, R.H., et al., *Prolonged space flight-induced alterations in the structure and function of human skeletal muscle fibres.* J Physiol, 2010. **588**(Pt 18): p. 3567-92.
127. Trappe, S., et al., *Exercise in space: human skeletal muscle after 6 months aboard the International Space Station.* J Appl Physiol (1985), 2009. **106**(4): p. 1159-68.
128. Genc, K.O., et al., *Foot forces during exercise on the International Space Station.* J Biomech, 2010. **43**(15): p. 3020-7.
129. Lee, S.M., et al., *Foot-ground reaction force during resistive exercise in parabolic flight.* Aviat Space Environ Med, 2004. **75**(5): p. 405-12.
130. Schneider, S.M., et al., *Training with the International Space Station interim resistive exercise device.* Med Sci Sports Exerc, 2003. **35**(11): p. 1935-45.
131. Smith, S.M., et al., *Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry.* J Bone Miner Res, 2012. **27**(9): p. 1896-906.
132. Stein, T.P., et al., *Protein kinetics during and after long-duration spaceflight on MIR.* Am J Physiol, 1999. **276**(6 Pt 1): p. E1014-21.
133. Smith, M., Zwart SR, Heer M, *Evidence Report: Risk Factor of Inadequate Nutrition.* 2015.
134. Johnston, R.S., Dietlein, L. F., *Biomedical Results from Skylab.* NTRS - NASA Technical Reports Server, 1977.
135. Zawadzki, J., T. Bober, and A. Siemienski, *Validity analysis of the Biodex System 3 dynamometer under static and isokinetic conditions.* Acta Bioeng Biomech, 2010. **12**(4): p. 25-32.
136. Sole, G., et al., *Test-retest reliability of isokinetic knee extension and flexion.* Arch Phys Med Rehabil, 2007. **88**(5): p. 626-31.
137. Ryder, J.W., et al., *Influence of muscle strength to weight ratio on functional task performance.* Eur J Appl Physiol, 2013. **113**(4): p. 911-21.
138. Laughlin, M.S., et al., *Functional Fitness Testing Results Following Long-Duration ISS Missions.* Aerosp Med Hum Perform, 2015. **86**(12 Suppl): p. A87-A91.
139. Grgic, J., et al., *Test-Retest Reliability of the One-Repetition Maximum (1RM) Strength Assessment: a Systematic Review.* Sports Med Open, 2020. **6**(1): p. 31.
140. Lee, S.M.C., et al., *Lean tissue mass and muscle strength: does resistive exercise prevent space flight deconditioning?* Med Sci Sports Exerc, 2004. **36**: p. S272.
141. Hackney, K.J., et al., *The Astronaut-Athlete: Optimizing Human Performance in Space.* J Strength Cond Res, 2015. **29**(12): p. 3531-45.
142. Dennis, S.C., T.D. Noakes, and A.N. Bosch, *Ventilation and blood lactate increase exponentially during incremental exercise.* J Sports Sci, 1992. **10**(5): p. 437-49.

143. Whipp, B.J., J.A. Davis, and K. Wasserman, *Ventilatory control of the 'isocapnic buffering'region in rapidly-incremental exercise.* Respiration physiology, 1989. **76**(3): p. 357-367.
144. Carriere, C., et al., *Anaerobic Threshold and Respiratory Compensation Point Identification During Cardiopulmonary Exercise Tests in Chronic Heart Failure.* Chest, 2019. **156**(2): p. 338-347.
145. Cannon, D.T., F.W. Kolkhorst, and M.J. Buono, *On the determination of ventilatory threshold and respiratory compensation point via respiratory frequency.* Int J Sports Med, 2009. **30**(3): p. 157-62.
146. Pallarés, J.G., et al., *Validity and reliability of ventilatory and blood lactate thresholds in well-trained cyclists.* PloS one, 2016. **11**(9).
147. Nattie, E. and A. Li, *Central chemoreceptors: locations and functions.* Compr Physiol, 2012. **2**(1): p. 221-54.
148. Beaver, W.L., K. Wasserman, and B.J. Whipp, *A new method for detecting anaerobic threshold by gas exchange.* J Appl Physiol (1985), 1986. **60**(6): p. 2020-7.
149. Kim, K.J., et al., *Novel Computerized Method for Automated Determination of Ventilatory Threshold and Respiratory Compensation Point.* Front Physiol, 2021. **12**: p. 782167.
150. Smith, S.M., et al., *Nutrition in space.* Nutr Today, 1997. **32**(1): p. 6-12.
151. Pavy-Le Traon, A., et al., *From space to Earth: advances in human physiology from 20 years of bed rest studies (1986-2006).* Eur J Appl Physiol, 2007. **101**(2): p. 143-94.
152. Fortney, S.M., V.S. Schneider, and J.E. Greenleaf, *The physiology of bed rest*, in *Handbook of Physiology, Environmental Physiology*. 1996, Am. Physiol. Soc.: Bethesda, MD. p. sect. 4, vol. II, chapt. 39, p. 889-939.
153. Convertino, V., et al., *Cardiovascular responses to exercise in middle-aged men after 10 days of bedrest.* Circulation, 1982. **65**(1): p. 134-40.
154. Adams, G.R., V.J. Caiozzo, and K.M. Baldwin, *Skeletal muscle unweighting: spaceflight and ground-based models.* J Appl Physiol (1985), 2003. **95**(6): p. 2185-201.
155. Buckey, J.C., *Muscle Loss: A Practical Guide to Maintaining Strength*, in *Space Physiology*. 2006, Oxford University Press: New York. p. 78-100.
156. Paddon-Jones, D., et al. *Increased protein catabolism following inactivity and chronic hypercortisolemia*. 2005.
157. Cavanagh, P.R., et al., *A novel lunar bed rest analogue.* Aviat Space Environ Med, 2013. **84**(11): p. 1191-5.
158. Andersen, P. and B. Saltin, *Maximal perfusion of skeletal muscle in man.* J Physiol, 1985. **366**: p. 233-49.
159. Noakes, T.D., *High VO2MAX with no history of training is due to high blood volume: an alternative explanation.* Br J Sports Med, 2005. **39**(8): p. 578.
160. Rowell, L.B., *Circulatory adjustments to dynamic exercise*, in *Human Circulation Regulation during Physical Stress*. 1986, Oxford University Press: New York. p. 213-256.
161. Saltin, B., *Hemodynamic adaptations to exercise.* Am J Cardiol, 1985. **55**(10): p. 42D-47D.
162. Saltin, B., J.A.L. Calbet, and P.D. Wagner, *Point/Counterpoint: In health and in a normoxic environment, VO2 max is/is not limited primarily by cardiac output and locomotor muscle blood flow.* J Appl Physiol, 2006. **100**(2): p. 744-748.
163. Saltin, B., et al., *Response to exercise after bed rest and after training.* Circulation, 1968. **38**(5 Suppl): p. VII1-78.
164. Hung, J., et al., *Mechanisms for decreased exercise capacity after bed rest in normal middle-aged men.* Am J Cardiol, 1983. **51**(2): p. 344-8.
165. Capelli, C., et al., *Factors determining the time course of VO2(max) decay during bedrest: implications for VO2(max) limitation.* Eur J Appl Physiol, 2006. **98**(2): p. 152-60.

166. Ferretti, G., et al., *The interplay of central and peripheral factors in limiting maximal O2 consumption in man after prolonged bed rest.* J Physiol, 1997. **501 (Pt 3)**: p. 677-86.
167. Convertino, V.A., *Exercise and adaptation to microgravity environments*, in *Handbook of Physiology, Environmental Physiology*. 1996, Am. Physiol. Soc.: Bethesda, MD. p. sect. 4, vol. II, chapt. 36, p. 815-843.
168. Nixon, J.V., et al., *Early cardiovascular adaptation to simulated zero gravity.* J Appl Physiol Respir Environ Exerc Physiol, 1979. **46**(3): p. 541-8.
169. Stremel, R.W., et al., *Cardiorespiratory deconditioning with static and dynamic leg exercise during bed rest.* J Appl Physiol, 1976. **41**(6): p. 905-9.
170. Ferretti, G., et al., *Effects of prolonged bed rest on cardiovascular oxygen transport during submaximal exercise in humans.* Eur J Appl Physiol Occup Physiol, 1998. **78**(5): p. 398-402.
171. Spaak, J., et al., *Long-term bed rest-induced reductions in stroke volume during rest and exercise: cardiac dysfunction vs. volume depletion.* J Appl Physiol (1985), 2005. **98**(2): p. 648-54.
172. Sundblad, P., J. Spaak, and D. Linnarsson, *Cardiovascular responses to upright and supine exercise in humans after 6 weeks of head-down tilt (-6 degrees).* Eur J Appl Physiol, 2000. **83**(4 -5): p. 303-9.
173. Atkov, O., V.S. Bednenko, and G.A. Fomina, *Ultrasound techniques in space medicine.* Aviat Space Environ Med, 1987. **58**(9 Pt 2): p. A69-73.
174. Dorfman, T.A., et al., *Cardiac atrophy in women following bed rest.* J Appl Physiol (1985), 2007. **103**(1): p. 8-16.
175. Dorfman, T.A., et al., *Diastolic suction is impaired by bed rest: MRI tagging studies of diastolic untwisting.* J Appl Physiol (1985), 2008. **104**(4): p. 1037-44.
176. Perhonen, M.A., et al., *Cardiac atrophy after bed rest and spaceflight.* J Appl Physiol (1985), 2001. **91**(2): p. 645-53.
177. Levine, B.D., J.H. Zuckerman, and J.A. Pawelczyk, *Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance.* Circulation, 1997. **96**(2): p. 517-25.
178. Perhonen, M.A., et al., *Cardiac atrophy after bed rest and spaceflight.* J Appl Physiol, 2001. **91**(2): p. 645-53.
179. Dorfman, T.A., et al., *Cardiac atrophy in women following bed rest.* J Appl Physiol, 2007. **103**(1): p. 8-16.
180. Hastings, J.L., et al., *Effect of rowing ergometry and oral volume loading on cardiovascular structure and function during bed rest.* J Appl Physiol (1985), 2012. **112**(10): p. 1735-43.
181. Shibata, S., M. Perhonen, and B.D. Levine, *Supine cycling plus volume loading prevent cardiovascular deconditioning during bed rest.* J Appl Physiol (1985), 2010. **108**(5): p. 1177-86.
182. Blomqvist, C.G., *Cardiovascular adaptation to weightlessness.* Med Sci Sports Exerc, 1983. **15**(5): p. 428-31.
183. Greenleaf, J.E., et al., *Effects of exercise on fluid exchange and body composition in man during 14-day bed rest.* J Appl Physiol Respir Environ Exerc Physiol, 1977. **43**(1): p. 126-32.
184. Blomqvist, C.G., et al., *Early cardiovascular adaptation to zero gravity simulated by head-down tilt.* Acta Astronaut, 1980. **7**(4-5): p. 543-53.
185. Engelke, K.A. and V.A. Convertino, *Restoration of peak vascular conductance after simulated microgravity by maximal exercise.* Clin Physiol, 1998. **18**(6): p. 544-53.
186. Convertino, V.A., et al., *Induced venous pooling and cardiorespiratory responses to exercise after bed rest.* J Appl Physiol, 1982. **52**(5): p. 1343-8.

187. Lee, S.M., et al., *LBNP exercise protects aerobic capacity and sprint speed of female twins during 30 days of bed rest.* J Appl Physiol (1985), 2009. **106**(3): p. 919-28.
188. Lee, S.M., et al., *LBNP exercise protects aerobic capacity and sprint speed of female twins during 30 days of bed rest.* J Appl Physiol, 2009. **106**(3): p. 919-28.
189. Convertino, V.A., et al., *Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity.* Aviat Space Environ Med, 1989. **60**(7): p. 653-8.
190. Savilov, A.A., V.I. Lobachik, and A.M. Babin, *Cardiovascular function of man exposed to LBNP tests.* Physiologist, 1990. **33**(1 Suppl): p. S128-32.
191. Fischer, D., et al., *Altered hormonal regulation and blood flow distribution with cardiovascular deconditioning after short-duration head down bed rest.* J Appl Physiol (1985), 2007. **103**(6): p. 2018-25.
192. Convertino, V.A. and W.H. Cooke, *Relationship between stroke volume and sympathetic nerve activity: new insights about autonomic mechanisms of syncope.* J Gravit Physiol, 2002. **9**(1): p. P63-6.
193. Engelke, K.A., et al., *Application of acute maximal exercise to protect orthostatic tolerance after simulated microgravity.* Am J Physiol, 1996. **271**(4 Pt 2): p. R837-47.
194. Convertino, V.A., *G-factor as a tool in basic research: mechanisms of orthostatic tolerance.* J Gravit Physiol, 1999. **6**(1): p. P73-6.
195. Engelke, K.A. and V.A. Convertino, *Catecholamine response to maximal exercise following 16 days of simulated microgravity.* Aviat Space Environ Med, 1996. **67**(3): p. 243-7.
196. Convertino, V.A., S.A. Bloomfield, and J.E. Greenleaf, *An overview of the issues: physiological effects of bed rest and restricted physical activity.* Med Sci Sports Exerc, 1997. **29**(2): p. 187-90.
197. Smorawinski, J., et al., *Effects of 3-day bed rest on physiological responses to graded exercise in athletes and sedentary men.* J Appl Physiol (1985), 2001. **91**(1): p. 249-57.
198. Convertino, V.A., D.J. Goldwater, and H. Sandler, *Effect of orthostatic stress on exercise performance after bedrest.* Aviat Space Environ Med, 1982. **53**(7): p. 652-7.
199. Scianowski, J., J. Kedziora, and K. Zolynski, *Red blood cell metabolism in men during long term bed rest.* Int J Occup Med Environ Health, 1995. **8**(4): p. 315-19.
200. Ferretti, G., *The effect of prolonged bed rest on maximal instantaneous muscle power and its determinants.* Int J Sports Med, 1997. **18 Suppl 4**: p. S287-9.
201. Blamick, C.A., D.J. Goldwater, and V.A. Convertino, *Leg vascular responsiveness during acute orthostasis following simulated weightlessness.* Aviat Space Environ Med, 1988. **59**(1): p. 40-3.
202. Snell, P.G., et al., *Maximal vascular leg conductance in trained and untrained men.* J Appl Physiol (1985), 1987. **62**(2): p. 606-10.
203. Convertino, V.A., D.F. Doerr, and S.L. Stein, *Changes in size and compliance of the calf after 30 days of simulated microgravity.* J Appl Physiol (1985), 1989. **66**(3): p. 1509-12.
204. Kosmas, E.N., et al., *Relationship of peak exercise capacity with indexes of peripheral muscle vasodilation.* Med Sci Sports Exerc, 1996. **28**(10): p. 1254-9.
205. Martin, W.H., 3rd, et al., *Effects of aging, gender, and physical training on peripheral vascular function.* Circulation, 1991. **84**(2): p. 654-64.
206. Reading, J.L., et al., *Vascular conductance and aerobic power in sedentary and active subjects and heart failure patients.* J Appl Physiol (1985), 1993. **74**(2): p. 567-73.
207. Snell, P.G., et al., *Maximal vascular leg conductance in trained and untrained men.* J Appl Physiol, 1987. **62**(2): p. 606-10.
208. Hikida, R.S., et al., *Structural and metabolic characteristics of human skeletal muscle following 30 days of simulated microgravity.* Aviat Space Environ Med, 1989. **60**(7): p. 664-70.

209. Schneider, S.M., et al., *Lower-body negative-pressure exercise and bed-rest-mediated orthostatic intolerance.* Med Sci Sports Exerc, 2002. **34**(9): p. 1446-53.
210. Watenpaugh, D.E., et al., *Lower body negative pressure (LBNP) exercise plus brief post-exercise LBNP improves post-bed rest orthostatic tolerance.* J Appl Physiol, 2007.
211. Koryak, Y., *Effect of 120 days of bed-rest with and without countermeasures on the mechanical properties of the triceps surae muscle in young women.* Eur J Appl Physiol Occup Physiol, 1998. **78**(2): p. 128-35.
212. Convertino, V.A., *Effects of Deconditioning and Reconditioning on Aerobic Power*, in *Deconditioning and Reconditioning*, J.E. Greenleaf, Editor. 2004, CRC Press: Washington, D.C. p. 13-25.
213. Greenleaf, J.E., et al., *Work capacity during 30 days of bed rest with isotonic and isokinetic exercise training.* J Appl Physiol (1985), 1989. **67**(5): p. 1820-6.
214. Convertino, V.A., *Clinical aspects of the control of plasma volume at microgravity and during return to one gravity.* Med Sci Sports Exerc, 1996. **28**(10 Suppl): p. S45-52.
215. Convertino, V.A., D.J. Goldwater, and H. Sandler, *Oxygen uptake kinetics of constant-load work: upright vs. supine exercise.* Aviat Space Environ Med, 1984. **55**(6): p. 501-6.
216. Caiani, E.G., et al., *Effects of 5 days of head-down bed rest, with and without short-arm centrifugation as countermeasure, on cardiac function in males (BR-AG1 study).* J Appl Physiol (1985), 2014. **117**(6): p. 624-32.
217. Zhang, L.F., *Vascular adaptation to microgravity: what have we learned?* J Appl Physiol, 2001. **91**(6): p. 2415-30.
218. Berg, H.E., et al., *Work capacity and metabolic and morphologic characteristics of the human quadriceps muscle in response to unloading.* Clin Physiol, 1993. **13**(4): p. 337-47.
219. Yang, Y., et al., *Hypergravity resistance exercise: the use of artificial gravity as potential countermeasure to microgravity.* J Appl Physiol, 2007. **103**(5): p. 1879-87.
220. Taylor, H.L., A. Henschel, and et al., *Effects of bed rest on cardiovascular function and work performance.* J Appl Physiol, 1949. **2**(5): p. 223-39.
221. Convertino, V.A., D.J. Goldwater, and H. Sandler, *Bedrest-induced peak VO2 reduction associated with age, gender, and aerobic capacity.* Aviat Space Environ Med, 1986. **57**(1): p. 17-22.
222. Convertino, V.A., et al., *Cardiorespiratory responses to exercise after bed rest in men and women.* Acta Astronaut, 1977. **4**(7-8): p. 895-905.
223. Greenleaf, J.E. and S. Kozlowski, *Reduction in peak oxygen uptake after prolonged bed rest.* Med Sci Sports Exerc, 1982. **14**(6): p. 477-80.
224. DeBusk, R.F., et al., *Exercise conditioning in middle-aged men after 10 days of bed rest.* Circulation, 1983. **68**(2): p. 245-50.
225. Caiozzo, V.J., et al., *A comparison of gas exchange indices used to detect the anaerobic threshold.* J Appl Physiol, 1982. **53**(5): p. 1184-9.
226. Convertino, V.A., *Exercise as a countermeasure for physiological adaptation to prolonged spaceflight.* Med Sci Sports Exerc, 1996. **28**(8): p. 999-1014.
227. Katayama, K., et al., *Ventilatory and cardiovascular responses to hypercapnia after 20 days of head-down bed rest.* Aviat Space Environ Med, 2004. **75**(4): p. 312-6.
228. Lee, S.M., et al., *Upright exercise or supine lower body negative pressure exercise maintains exercise responses after bed rest.* Med Sci Sports Exerc, 1997. **29**(7): p. 892-900.
229. Mujika, I. and S. Padilla, *Detraining: loss of training-induced physiological and performance adaptations. Part II: Long term insufficient training stimulus.* Sports Med, 2000. **30**(3): p. 145-54.
230. Convertino, V.A., et al., *Effect of simulated weightlessness on exercise-induced anaerobic threshold.* Aviat Space Environ Med, 1986. **57**(4): p. 325-31.

231. Sullivan, M.J., et al., *Prevention of bedrest-induced physical deconditioning by daily dobutamine infusions. Implications for drug-induced physical conditioning.* J Clin Invest, 1985. **76**(4): p. 1632-42.
232. Williams, D.A. and V.A. Convertino, *Circulating lactate and FFA during exercise: effect of reduction in plasma volume following exposure to simulated microgravity.* Aviat Space Environ Med, 1988. **59**(11 Pt 1): p. 1042-6.
233. Tabata, I., et al., *Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest.* J Appl Physiol (1985), 1999. **86**(3): p. 909-14.
234. Woodman, C.R., L.A. Sebastian, and C.M. Tipton, *Influence of simulated microgravity on cardiac output and blood flow distribution during exercise.* J Appl Physiol (1985), 1995. **79**(5): p. 1762-8.
235. Fortney, S.M., *Exercise thermoregulation: possible effects of spaceflight.* SAE Tech Paper Series, 1991. **911460**: p. 1.
236. Fortney, S.M. *Exercise thermoregulation: possible effects of spaceflight*. in *SAE International, 21st International Conference on Environmental Systems*. 1991. San Francisco, CA.
237. Pisacane, V.L., et al., *Thermoregulatory models of space shuttle and space station activities.* Aviat Space Environ Med, 2007. **78**(4 Suppl): p. A48-55.
238. Greenleaf, J.E., *Energy and thermal regulation during bed rest and spaceflight.* J Appl Physiol (1985), 1989. **67**(2): p. 507-16.
239. Lee, S.M., W.J. Williams, and S.M. Schneider, *Role of skin blood flow and sweating rate in exercise thermoregulation after bed rest.* J Appl Physiol, 2002. **92**(5): p. 2026-34.
240. Greenleaf, J.E. and R.D. Reese, *Exercise thermoregulation after 14 days of bed rest.* J Appl Physiol Respir Environ Exerc Physiol, 1980. **48**(1): p. 72-8.
241. Pandolf, K.B., et al., *Predicting human heat strain and performance with application to space operations.* Aviat Space Environ Med, 1995. **66**(4): p. 364-8.
242. Rimmer, D.W., et al., *Efficacy of liquid cooling garments to minimize heat strain during Space Shuttle deorbit and landing.* Medicine and Science in Sports and Exercise, 1999. **31**: p. S305.
243. Leach, C.S., et al., *Regulation of body fluid compartments during short-term spaceflight.* J Appl Physiol (1985), 1996. **81**(1): p. 105-16.
244. Novak, L., *Our experience in the evaluation of the thermal comfort during the space flight and in the simulated space environment.* Acta Astronaut, 1991. **23**: p. 179-86.
245. Crandall, C.G., et al., *Prolonged head-down tilt exposure reduces maximal cutaneous vasodilator and sweating capacity in humans.* J Appl Physiol (1985), 2003. **94**(6): p. 2330-6.
246. Michikami, D., et al., *Attenuated thermoregulatory sweating and cutaneous vasodilation after 14-day bed rest in humans.* J Appl Physiol (1985), 2004. **96**(1): p. 107-14.
247. Fortney, S.M., *Thermoregulatory adaptations to inactivity*, in *Adaptive Physiology to Stressful Environments*. 1987, CRC Press: Boca Raton, FL. p. 75-84.
248. Greenleaf, J.E. and R.D. Reese, *Exercise thermoregulation after 14 days of bed rest.* J Appl Physiol, 1980. **48**(1): p. 72-8.
249. Ertl, A.C., et al., *Exercise thermoregulation in men after 1 and 24-hours of 6 degrees head-down tilt.* Aviat Space Environ Med, 2000. **71**(2): p. 150-5.
250. Greenleaf, J.E., *Exercise thermoregulation with bed rest, confinement, and immersion deconditioning.* Ann N Y Acad Sci, 1997. **813**: p. 741-50.
251. Shibasaki, M., et al., *Exercise throughout 6 degrees head-down tilt bed rest preserves thermoregulatory responses.* J Appl Physiol (1985), 2003. **95**(5): p. 1817-23.
252. Stuart, C.A., et al., *Effect of dietary protein on bed-rest-related changes in whole-body-protein synthesis.* Am J Clin Nutr, 1990. **52**(3): p. 509-14.

253. Ferrando, A.A., et al., *Magnetic resonance imaging quantitation of changes in muscle volume during 7 days of strict bed rest.* Aviat Space Environ Med, 1995. **66**(10): p. 976-81.
254. Dudley, G.A., et al., *Alterations of the in vivo torque-velocity relationship of human skeletal muscle following 30 days exposure to simulated microgravity.* Aviat Space Environ Med, 1989. **60**(7): p. 659-63.
255. Dudley, G.A., et al., *Adaptations to unilateral lower limb suspension in humans.* Aviat Space Environ Med, 1992. **63**(8): p. 678-83.
256. Katkovskiy, B.S., Machinskiy, G.V., Toman, P.S., Danilova, V.I., Demida, B.F., *Man's physical performance after 30-day hypokinesia with countermeasures.* Kosm Biol Aviakosm Med, 1974. **8**: p. 43-47.
257. Berg, H.E., et al., *Effects of lower limb unloading on skeletal muscle mass and function in humans.* J Appl Physiol (1985), 1991. **70**(4): p. 1882-5.
258. Hather, B.M., et al., *Skeletal muscle responses to lower limb suspension in humans.* J Appl Physiol (1985), 1992. **72**(4): p. 1493-8.
259. Grigoryeva, L.S., Kozlovskaya I.B., *Effects of 7-day immersion hypokinesia on characteristics of precision of movements.* Kosm Biol Aviakosm Med, 1985. **19**: p. 38-42.
260. Koryak, Y., *"DRY" immersion induces neural and contractile adaptations in the human triceps surae muscle.* Environ Med, 2002. **46**(1-2): p. 17-27.
261. Kozlovskaya, I.B., Aslanova, I.F., Barmin, V.A., et al., *The nature and characteristics of gravitational ataxia.* Physiologist, 1983. **510**(Pt. 1): p. 287-295.
262. Kozlovskaya, I.B., et al., *Experimental analysis of motor effects of weightlessness.* Physiologist, 1982. **25**(6): p. S49-52.
263. Miller, T.F., et al., *Effect of mechanical stimulation of the support zones of soles on the muscle stiffness in 7-day dry immersion.* J Gravit Physiol, 2004. **11**(2): p. P135-6.
264. Netreba, A.I., et al., *Effect of dry immersion in combination with stimulation of foot support zones upon muscle force-velocity characteristics.* J Gravit Physiol, 2004. **11**(2): p. P129-30.
265. Shenkman, B.S., et al., *Afferent and peripheral control of muscle fiber properties during gravitational unloading.* J Gravit Physiol, 2004. **11**(2): p. P111-4.
266. Alkner, B.A. and P.A. Tesch, *Efficacy of a gravity-independent resistance exercise device as a countermeasure to muscle atrophy during 29-day bed rest.* Acta Physiol Scand, 2004. **181**(3): p. 345-57.
267. Akima, H., et al., *Leg-press resistance training during 20 days of 6 degrees head-down-tilt bed rest prevents muscle deconditioning.* Eur J Appl Physiol, 2000. **82**(1-2): p. 30-8.
268. Shackelford, L.C., et al., *Resistance exercise as a countermeasure to disuse-induced bone loss.* J Appl Physiol (1985), 2004. **97**(1): p. 119-29.
269. Berg, H.E., et al., *Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity.* Eur J Appl Physiol, 2007. **99**(3): p. 283-9.
270. Moritani, T. and H.A. deVries, *Neural factors versus hypertrophy in the time course of muscle strength gain.* Am J Phys Med, 1979. **58**(3): p. 115-30.
271. Koryak, Y., *Contractile properties of the human triceps surae muscle during simulated weightlessness.* Eur J Appl Physiol Occup Physiol, 1995. **70**(4): p. 344-50.
272. Berg, H.E., L. Larsson, and P.A. Tesch, *Lower limb skeletal muscle function after 6 wk of bed rest.* J Appl Physiol (1985), 1997. **82**(1): p. 182-8.
273. Grana, E.A., F. Chiou-Tan, and M.M. Jaweed, *Endplate dysfunction in healthy muscle following a period of disuse.* Muscle Nerve, 1996. **19**(8): p. 989-93.
274. de Boer, M.D., et al., *Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men.* J Physiol, 2007. **583**(Pt 3): p. 1079-91.

275. Mulder, E.R., et al., *High-density surface EMG study on the time course of central nervous and peripheral neuromuscular changes during 8 weeks of bed rest with or without resistive vibration exercise.* J Electromyogr Kinesiol, 2009. **19**(2): p. 208-18.
276. Cescon, C. and M. Gazzoni, *Short term bed-rest reduces conduction velocity of individual motor units in leg muscles.* J Electromyogr Kinesiol, 2010. **20**(5): p. 860-7.
277. de Boer, M.D., et al., *The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse.* J Physiol, 2007. **585**(Pt 1): p. 241-51.
278. Glover, E.I., et al., *Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion.* J Physiol, 2008. **586**(24): p. 6049-61.
279. Paddon-Jones, D., et al., *Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress.* J Clin Endocrinol Metab, 2006. **91**(12): p. 4836-41.
280. Symons, T.B., et al., *Artificial gravity maintains skeletal muscle protein synthesis during 21 days of simulated microgravity.* J Appl Physiol (1985), 2009. **107**(1): p. 34-8.
281. Ferrando, A.A., et al., *Resistance exercise maintains skeletal muscle protein synthesis during bed rest.* J Appl Physiol (1985), 1997. **82**(3): p. 807-10.
282. Dickinson, J.M., et al., *Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids.* J Nutr, 2011. **141**(5): p. 856-62.
283. Drummond, M.J., et al., *Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults.* Am J Physiol Endocrinol Metab, 2012. **302**(9): p. E1113-22.
284. Tesch, P.A., et al., *Skeletal muscle proteolysis in response to short-term unloading in humans.* J Appl Physiol (1985), 2008. **105**(3): p. 902-6.
285. Attaix, D., V.E. Baracos, and C. Pichard, *Muscle wasting: a crosstalk between protein synthesis and breakdown signalling.* Curr Opin Clin Nutr Metab Care, 2012. **15**(3): p. 209-10.
286. Marimuthu, K., A.J. Murton, and P.L. Greenhaff, *Mechanisms regulating muscle mass during disuse atrophy and rehabilitation in humans.* J Appl Physiol (1985), 2011. **110**(2): p. 555-60.
287. Glover, E.I., et al., *Little change in markers of protein breakdown and oxidative stress in humans in immobilization-induced skeletal muscle atrophy.* Appl Physiol Nutr Metab, 2010. **35**(2): p. 125-33.
288. Berg, H.E., *Effects of unloading on skeletal muscle mass and function in man.* 1997, Karolinska Institute.: Stockholm.
289. Il'ina-Kakuyeva, Y.I., Portugalov, V.V., Krivenkov, N.P. et al., *Effects of physical conditioning and electric stimulation on metabolic processes in the soleus muscle and structure thereof in hypokinetic man.* Kosm Biol Aviakosm Med, 1979. **13**: p. 35-38.
290. Hortobagyi, T., et al., *Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans.* J Physiol, 2000. **524 Pt 1**: p. 293-304.
291. Rudnick, J., et al., *Differential expression of nitric oxide synthases (NOS 1-3) in human skeletal muscle following exercise countermeasure during 12 weeks of bed rest.* FASEB J, 2004. **18**(11): p. 1228-30.
292. Bamman, M.M., et al., *Resistance exercise prevents plantar flexor deconditioning during bed rest.* Med Sci Sports Exerc, 1997. **29**(11): p. 1462-8.
293. Bamman, M.M., et al., *Enhanced protein electrophoresis technique for separating human skeletal muscle myosin heavy chain isoforms.* Electrophoresis, 1999. **20**(3): p. 466-8.

294. Gallagher, P., et al., *Effects of 84-days of bedrest and resistance training on single muscle fibre myosin heavy chain distribution in human vastus lateralis and soleus muscles.* Acta Physiol Scand, 2005. **185**(1): p. 61-9.
295. Trappe, S., et al., *Human single muscle fibre function with 84 day bed-rest and resistance exercise.* J Physiol, 2004. **557**(Pt 2): p. 501-13.
296. Brocca, L., et al., *The time course of the adaptations of human muscle proteome to bed rest and the underlying mechanisms.* J Physiol, 2012. **590**(20): p. 5211-30.
297. Virtanen, P., et al., *Effect of immobilization on carbonic anhydrase III and myoglobin content in human leg muscle.* Acta Physiol Scand, 1991. **142**(3): p. 303-6.
298. Ward, G.R., et al., *Activation of human muscle pyruvate dehydrogenase with activity and immobilization.* Clin Sci (Lond), 1986. **70**(2): p. 207-10.
299. Dolkas, C.B. and J.E. Greenleaf, *Insulin and glucose responses during bed rest with isotonic and isometric exercise.* J Appl Physiol Respir Environ Exerc Physiol, 1977. **43**(6): p. 1033-8.
300. Lipman, R.L., et al., *Glucose intolerance during decreased physical activity in man.* Diabetes, 1972. **21**(2): p. 101-7.
301. Stuart, C.A., et al., *Bed-rest-induced insulin resistance occurs primarily in muscle.* Metabolism, 1988. **37**(8): p. 802-6.
302. Brooks, N., et al., *Resistance training and timed essential amino acids protect against the loss of muscle mass and strength during 28 days of bed rest and energy deficit.* J Appl Physiol (1985), 2008. **105**(1): p. 241-8.
303. Biolo, G., et al., *Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest.* Am J Clin Nutr, 2007. **86**(2): p. 366-72.
304. Biolo, G., et al., *Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest.* Am J Clin Nutr, 2008. **88**(4): p. 950-8.
305. Stein, T.P., et al., *Branched-chain amino acid supplementation during bed rest: effect on recovery.* J Appl Physiol (1985), 2003. **94**(4): p. 1345-52.
306. Stein, T.P. and S. Blanc, *Does protein supplementation prevent muscle disuse atrophy and loss of strength?* Crit Rev Food Sci Nutr, 2011. **51**(9): p. 828-34.
307. Wolfe, R.R., S.L. Miller, and K.B. Miller, *Optimal protein intake in the elderly.* Clin Nutr, 2008. **27**(5): p. 675-84.
308. Mamerow, M.M., et al., *Dietary protein distribution positively influences 24 h muscle protein synthesis.* J Nutr, 2013: p. in review.
309. Devkota, S. and D.K. Layman, *Protein metabolic roles in treatment of obesity.* Curr Opin Clin Nutr Metab Care, 2010. **13**(4): p. 403-7.
310. Wall, B.T., et al., *Leucine co-ingestion improves post-prandial muscle protein accretion in elderly men.* Clin Nutr, 2013. **32**(3): p. 412-9.
311. Churchward-Venne, T.A., et al., *Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men.* J Physiol, 2012. **590**(11): p. 2751-65.
312. Katsanos, C.S., et al., *Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids.* Am J Clin Nutr, 2005. **82**(5): p. 1065-73.
313. Katsanos, C.S., et al., *A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly.* Am J Physiol Endocrinol Metab, 2006. **291**(2): p. E381-7.
314. Rieu, I., et al., *Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia.* J Physiol, 2006. **575**(Pt 1): p. 305-15.
315. Cuthbertson, D., et al., *Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle.* FASEB J, 2005. **19**(3): p. 422-4.

316. Fujita, S. and E. Volpi, *Amino acids and muscle loss with aging.* J Nutr, 2006. **136**(1 Suppl): p. 277S-80S.
317. Dillon, E.L., *Nutritionally essential amino acids and metabolic signaling in aging.* Amino Acids, 2013. **45**(3): p. 431-41.
318. Wall, B.T., et al., *Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men.* J Clin Endocrinol Metab, 2013. **98**(12): p. 4872-81.
319. Res, P.T., et al., *Protein ingestion before sleep improves postexercise overnight recovery.* Med Sci Sports Exerc, 2012. **44**(8): p. 1560-9.
320. Astrand, I., *Aerobic work capacity in men and women with special reference to age.* Acta Physiol Scand Suppl, 1960. **49**(169): p. 1-92.
321. Frontera, W.R., et al., *Aging of skeletal muscle: a 12-yr longitudinal study.* J Appl Physiol (1985), 2000. **88**(4): p. 1321-6.
322. Fleg, J.L. and J. Strait, *Age-associated changes in cardiovascular structure and function: a fertile milieu for future disease.* Heart Fail Rev, 2012. **17**(4-5): p. 545-54.
323. Fleg, J.L. and E.G. Lakatta, *Role of muscle loss in the age-associated reduction in VO2 max.* J Appl Physiol (1985), 1988. **65**(3): p. 1147-51.
324. Kortebein, P., et al., *Functional impact of 10 days of bed rest in healthy older adults.* J Gerontol A Biol Sci Med Sci, 2008. **63**(10): p. 1076-81.
325. English, K.L. and D. Paddon-Jones, *Protecting muscle mass and function in older adults during bed rest.* Curr Opin Clin Nutr Metab Care, 2010. **13**(1): p. 34-9.
326. Science, U.S.C.H.C.o. and Astronautics, *Qualifications for Astronauts: Hearings Before the Special Subcommittee on the Selection of Astronauts of the Committee on Science and Astronauts, U. S. House of Representatives; Eighty-seventh Congress, Second Session, July 17 and 18, 1962*. 1962: U.S. Government Printing Office.
327. *Artemis Plan: NASA's Lunar Exploration Program Overview*, N.A.a.S. Administration, Editor. 2020.
328. Wright, N.C., et al., *The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine.* J Bone Miner Res, 2014. **29**(11): p. 2520-6.
329. Looker, A.C., et al., *Trends in osteoporosis and low bone mass in older US adults, 2005-2006 through 2013-2014.* Osteoporos Int, 2017. **28**(6): p. 1979-1988.
330. Alswat, K.A., *Gender Disparities in Osteoporosis.* J Clin Med Res, 2017. **9**(5): p. 382-387.
331. Colafella, K.M.M. and K.M. Denton, *Sex-specific differences in hypertension and associated cardiovascular disease.* Nat Rev Nephrol, 2018. **14**(3): p. 185-201.
332. Honda, Y., et al., *Spaceflight and ageing: reflecting on Caenorhabditis elegans in space.* Gerontology, 2014. **60**(2): p. 138-42.
333. De Souza, M.J., et al., *2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013.* Br J Sports Med, 2014. **48**(4): p. 289.
334. Fredericson, M., et al., *The Male Athlete Triad-A Consensus Statement From the Female and Male Athlete Triad Coalition Part II: Diagnosis, Treatment, and Return-To-Play.* Clin J Sport Med, 2021. **31**(4): p. 349-366.
335. Wiebe, D.J., et al., *The Male Athlete Triad-A Consensus Statement From the Female and Male Athlete Triad Coalition Part 1: Definition and Scientific Basis.* Clin J Sport Med, 2021. **31**(4): p. 345-353.
336. Zwart, S.R., et al., *Body mass changes during long-duration spaceflight.* Aviat Space Environ Med, 2014. **85**(9): p. 897-904.
337. Reed, B.G. and B.R. Carr, *The Normal Menstrual Cycle and the Control of Ovulation*, in *Endotext*, K.R. Feingold, et al., Editors. 2000, MDText.com, Inc.: South Dartmouth (MA).

338. Khosla, S. and D.G. Monroe, *Regulation of Bone Metabolism by Sex Steroids.* Cold Spring Harb Perspect Med, 2018. **8**(1).
339. Markle, J.G. and E.N. Fish, *SeXX matters in immunity.* Trends Immunol, 2014. **35**(3): p. 97-104.
340. Oyola, M.G. and R.J. Handa, *Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity.* Stress, 2017. **20**(5): p. 476-494.
341. Cano Sokoloff, N., M. Misra, and K.E. Ackerman, *Exercise, Training, and the Hypothalamic-Pituitary-Gonadal Axis in Men and Women.* Front Horm Res, 2016. **47**: p. 27-43.
342. Medicine, A.C.o.S. and G. Liguori, *ACSM's Guidelines for Exercise Testing and Prescription*. 11th ed. ed. 2021, Philadelphia: Lippincott Williams & Wilkins.
343. Piercy, K.L., et al., *The Physical Activity Guidelines for Americans.* JAMA, 2018. **320**(19): p. 2020-2028.
344. Tremblay, M.S., J.L. Copeland, and W. Van Helder, *Influence of exercise duration on post-exercise steroid hormone responses in trained males.* Eur J Appl Physiol, 2005. **94**(5-6): p. 505-13.
345. Tremblay, M.S., J.L. Copeland, and W. Van Helder, *Effect of training status and exercise mode on endogenous steroid hormones in men.* J Appl Physiol (1985), 2004. **96**(2): p. 531-9.
346. Daly, W., et al., *Relationship between stress hormones and testosterone with prolonged endurance exercise.* Eur J Appl Physiol, 2005. **93**(4): p. 375-80.
347. Kraemer, W.J. and N.A. Ratamess, *Hormonal responses and adaptations to resistance exercise and training.* Sports Med, 2005. **35**(4): p. 339-61.
348. Nattiv, A., et al., *The Male Athlete Triad-A Consensus Statement From the Female and Male Athlete Triad Coalition Part 1: Definition and Scientific Basis.* Clin J Sport Med, 2021. **31**(4): p. 345-353.
349. Keizer, H., et al., *Changes in basal plasma testosterone, cortisol, and dehydroepiandrosterone sulfate in previously untrained males and females preparing for a marathon.* Int J Sports Med, 1989. **10 Suppl 3**: p. S139-45.
350. Hakimi, O. and L.C. Cameron, *Effect of Exercise on Ovulation: A Systematic Review.* Sports Med, 2017. **47**(8): p. 1555-1567.
351. Loucks, A.B., M. Verdun, and E.M. Heath, *Low energy availability, not stress of exercise, alters LH pulsatility in exercising women.* J Appl Physiol (1985), 1998. **84**(1): p. 37-46.
352. Williams, N.I., et al., *Magnitude of daily energy deficit predicts frequency but not severity of menstrual disturbances associated with exercise and caloric restriction.* Am J Physiol Endocrinol Metab, 2015. **308**(1): p. E29-39.
353. Amann, R.P., et al., *Effects of microgravity or simulated launch on testicular function in rats.* J Appl Physiol (1985), 1992. **73**(2 Suppl): p. 174S-185S.
354. Grindeland, R.E., et al., *Cosmos 1887 mission overview: effects of microgravity on rat body and adrenal weights and plasma constituents.* FASEB J, 1990. **4**(1): p. 105-9.
355. Smith, S.M., et al., *Long-duration space flight and bed rest effects on testosterone and other steroids.* J Clin Endocrinol Metab, 2012. **97**(1): p. 270-8.
356. Tou, J.C., R.E. Grindeland, and C.E. Wade, *Effects of diet and exposure to hindlimb suspension on estrous cycling in Sprague-Dawley rats.* Am J Physiol Endocrinol Metab, 2004. **286**(3): p. E425-33.
357. Holets, L.M., et al., *Spaceflight Inhibits Ovarian Follicle Development, Induces Down Regulation of Estrogen Receptor Alpha, and Alters Metabolic Pathways and Gene Expression in Mouse Uterus.* Biology of Reproduction, 2012. **87**(Suppl_1): p. 18-18.
358. Hong, X., et al., *Effects of spaceflight aboard the International Space Station on mouse estrous cycle and ovarian gene expression.* NPJ Microgravity, 2021. **7**(1): p. 11.

359. Sandler, H. and D.L. Winter, *Physiological responses of women to simulated weightlessness: a review of the first female bed-rest study.* 1978.
360. Rock, J.A. and S.M. Fortney, *Medical and surgical considerations for women in spaceflight.* Obstet Gynecol Surv, 1984. **39**(8): p. 525-35.
361. Jain, V. and V.E. Wotring, *Medically induced amenorrhea in female astronauts.* NPJ Microgravity, 2016. **2**: p. 16008.
362. Jennings, R.T. and E.S. Baker, *Gynecological and reproductive issues for women in space: a review.* Obstet Gynecol Surv, 2000. **55**(2): p. 109-16.
363. Steller, J.G., et al., *Gynecologic Risk Mitigation Considerations for Long-Duration Spaceflight.* Aerosp Med Hum Perform, 2020. **91**(7): p. 543-564.
364. *Committee Opinion No. 602: Depot medroxyprogesterone acetate and bone effects.* Obstet Gynecol, 2014. **123**(6): p. 1398-1402.
365. Cibula, D., et al., *Low-dose estrogen combined oral contraceptives may negatively influence physiological bone mineral density acquisition during adolescence.* Eur J Endocrinol, 2012. **166**(6): p. 1003-11.
366. Hartard, M., et al., *Comparison of the skeletal effects of the progestogens desogestrel and levonorgestrel in oral contraceptive preparations in young women: controlled, open, partly randomized investigation over 13 cycles.* Contraception, 2006. **74**(5): p. 367-75.
367. Scholes, D., et al., *Oral contraceptive use and bone density change in adolescent and young adult women: a prospective study of age, hormone dose, and discontinuation.* J Clin Endocrinol Metab, 2011. **96**(9): p. E1380-7.
368. Pasco, J.A., et al., *Oral contraceptives and bone mineral density: A population-based study.* Am J Obstet Gynecol, 2000. **182**(2): p. 265-9.
369. Prior, J.C., et al., *Oral contraceptive use and bone mineral density in premenopausal women: cross-sectional, population-based data from the Canadian Multicentre Osteoporosis Study.* CMAJ, 2001. **165**(8): p. 1023-9.
370. Berenson, A.B., et al., *Effects of hormonal contraception on bone mineral density after 24 months of use.* Obstet Gynecol, 2004. **103**(5 Pt 1): p. 899-906.
371. Polatti, F., et al., *Bone mass and long-term monophasic oral contraceptive treatment in young women.* Contraception, 1995. **51**(4): p. 221-4.
372. Baxter-Jones, A.D.G. and S.A. Jackowski, *Sex differences in bone mineral content and bone geometry accrual: a review of the Paediatric Bone Mineral Accural Study (1991-2017).* Ann Hum Biol, 2021. **48**(6): p. 503-516.
373. Gabel, L., H.M. Macdonald, and H.A. McKay, *Sex Differences and Growth-Related Adaptations in Bone Microarchitecture, Geometry, Density, and Strength From Childhood to Early Adulthood: A Mixed Longitudinal HR-pQCT Study.* J Bone Miner Res, 2017. **32**(2): p. 250-263.
374. Lauretani, F., et al., *Longitudinal changes in BMD and bone geometry in a population-based study.* J Bone Miner Res, 2008. **23**(3): p. 400-8.
375. Vico, L., et al., *Cortical and Trabecular Bone Microstructure Did Not Recover at Weight-Bearing Skeletal Sites and Progressively Deteriorated at Non-Weight-Bearing Sites During the Year Following International Space Station Missions.* J Bone Miner Res, 2017. **32**(10): p. 2010-2021.
376. LeBlanc, A., et al., *Bone mineral and lean tissue loss after long duration space flight.* J Musculoskelet Neuronal Interact, 2000. **1**(2): p. 157-60.
377. Zwart, S.R., et al., *Lower body negative pressure treadmill exercise as a countermeasure for bed rest-induced bone loss in female identical twins.* Bone, 2007. **40**(2): p. 529-37.
378. Beller, G., et al., *WISE-2005: bed-rest induced changes in bone mineral density in women during 60 days simulated microgravity.* Bone, 2011. **49**(4): p. 858-66.

379. Smith, S.M., et al., *WISE-2005: supine treadmill exercise within lower body negative pressure and flywheel resistive exercise as a countermeasure to bed rest-induced bone loss in women during 60-day simulated microgravity.* Bone, 2008. **42**(3): p. 572-81.
380. Armbrecht, G., et al., *Trabecular and cortical bone density and architecture in women after 60 days of bed rest using high-resolution pQCT: WISE 2005.* J Bone Miner Res, 2011. **26**(10): p. 2399-410.
381. Smith, S.M., et al., *Men and women in space: bone loss and kidney stone risk after long-duration spaceflight.* J Bone Miner Res, 2014. **29**(7): p. 1639-45.
382. Gabel, L., et al., *Incomplete recovery of bone strength and trabecular microarchitecture at the distal tibia 1 year after return from long duration spaceflight.* Sci Rep, 2022. **12**(1): p. 9446.
383. Johnston, R.S., L.F. Dietlein, and C.A. Berry, *Biomedical Results of Apollo*. 1975, Scientific and Technical Information Office, National Aeronautics and Space Administration.
384. Montalvo, A.M., et al., *"What's my risk of sustaining an ACL injury while playing sports?" A systematic review with meta-analysis.* Br J Sports Med, 2019. **53**(16): p. 1003-1012.
385. Silvers-Granelli, H., *Why Female Athletes Injure Their ACL's More Frequently? What can we do to mitigate their risk?* Int J Sports Phys Ther, 2021. **16**(4): p. 971-977.
386. Nedelec, E., et al., *Effect of menstrual cycle phase, menstrual irregularities and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a protocol for a systematic review and meta-analysis.* BMJ Open Sport Exerc Med, 2021. **7**(4): p. e001170.
387. Quatman, C.E. and T.E. Hewett, *The anterior cruciate ligament injury controversy: is "valgus collapse" a sex-specific mechanism?* Br J Sports Med, 2009. **43**(5): p. 328-35.
388. Balachandar, V., et al., *Effects of the menstrual cycle on lower-limb biomechanics, neuromuscular control, and anterior cruciate ligament injury risk: a systematic review.* Muscles Ligaments Tendons J, 2017. **7**(1): p. 136-146.
389. Hewett, T.E., B.T. Zazulak, and G.D. Myer, *Effects of the menstrual cycle on anterior cruciate ligament injury risk: a systematic review.* Am J Sports Med, 2007. **35**(4): p. 659-68.
390. Belanger, L., et al., *Anterior cruciate ligament laxity related to the menstrual cycle: an updated systematic review of the literature.* J Can Chiropr Assoc, 2013. **57**(1): p. 76-86.
391. Lee, H., et al., *Differences in anterior cruciate ligament elasticity and force for knee flexion in women: oral contraceptive users versus non-oral contraceptive users.* Eur J Appl Physiol, 2014. **114**(2): p. 285-94.
392. Yu, W.D., et al., *Effect of estrogen on cellular metabolism of the human anterior cruciate ligament.* Clin Orthop Relat Res, 1999(366): p. 229-38.
393. Samuelson, K., et al., *Limited Evidence Suggests a Protective Association Between Oral Contraceptive Pill Use and Anterior Cruciate Ligament Injuries in Females: A Systematic Review.* Sports Health, 2017. **9**(6): p. 498-510.
394. Huxley, V.H., *Sex and the cardiovascular system: the intriguing tale of how women and men regulate cardiovascular function differently.* Adv Physiol Educ, 2007. **31**(1): p. 17-22.
395. Aitken, M.L., et al., *Influence of body size and gender on control of ventilation.* J Appl Physiol (1985), 1986. **60**(6): p. 1894-9.
396. Orshal, J.M. and R.A. Khalil, *Gender, sex hormones, and vascular tone.* Am J Physiol Regul Integr Comp Physiol, 2004. **286**(2): p. R233-49.
397. Kneale, B.J., et al., *Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature.* J Am Coll Cardiol, 2000. **36**(4): p. 1233-8.
398. Barnett, S.R., et al., *Effects of age and gender on autonomic control of blood pressure dynamics.* Hypertension, 1999. **33**(5): p. 1195-200.

399. Fu, Q., et al., *Hemodynamics of orthostatic intolerance: implications for gender differences.* Am J Physiol Heart Circ Physiol, 2004. **286**(1): p. H449-57.
400. Buckey, J.C., Jr., et al., *Orthostatic intolerance after spaceflight.* J Appl Physiol (1985), 1996. **81**(1): p. 7-18.
401. Blaber, A.P., R.L. Bondar, and M.S. Kassam, *Heart rate variability and short duration spaceflight: relationship to post-flight orthostatic intolerance.* BMC Physiol, 2004. **4**: p. 6.
402. Blaber, A.P., et al., *Impairment of cerebral blood flow regulation in astronauts with orthostatic intolerance after flight.* Stroke, 2011. **42**(7): p. 1844-50.
403. Green, D.J., et al., *Sex differences in vascular endothelial function and health in humans: impacts of exercise.* Exp Physiol, 2016. **101**(2): p. 230-42.
404. Kannel, W.B., et al., *Menopause and risk of cardiovascular disease: the Framingham study.* Ann Intern Med, 1976. **85**(4): p. 447-52.
405. Williams, J.S., E.C. Dunford, and M.J. MacDonald, *Impact of the menstrual cycle on peripheral vascular function in premenopausal women: systematic review and meta-analysis.* Am J Physiol Heart Circ Physiol, 2020. **319**(6): p. H1327-H1337.
406. Wenner, M.M., et al., *Mechanisms contributing to low orthostatic tolerance in women: the influence of oestradiol.* J Physiol, 2013. **591**(9): p. 2345-55.
407. Harm, D.L., et al., *Invited review: gender issues related to spaceflight: a NASA perspective.* J Appl Physiol (1985), 2001. **91**(5): p. 2374-83.
408. Fritsch-Yelle, J.M., et al., *Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight.* J Appl Physiol (1985), 1996. **81**(5): p. 2134-41.
409. Waters, W.W., M.G. Ziegler, and J.V. Meck, *Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance.* J Appl Physiol (1985), 2002. **92**(2): p. 586-94.
410. Franke, W.D., et al., *Cardiovascular and autonomic responses to lower body negative pressure: do not explain gender differences in orthostatic tolerance.* Clin Auton Res, 2003. **13**(1): p. 36-44.
411. Edgell, H., et al., *Cardiovascular responses to lower body negative pressure before and after 4 h of head-down bed rest and seated control in men and women.* J Appl Physiol (1985), 2012. **113**(10): p. 1604-12.
412. Frey, M.A., K.L. Mathes, and G.W. Hoffler, *Cardiovascular responses of women to lower body negative pressure.* Aviat Space Environ Med, 1986. **57**(6): p. 531-8.
413. Siamwala, J.H., et al., *Gender differences in tibial microvascular flow responses to head down tilt and lower body negative pressure.* Physiol Rep, 2017. **5**(4).
414. Custaud, M.A., et al., *Orthostatic tolerance and spontaneous baroreflex sensitivity in men versus women after 7 days of head-down bed rest.* Auton Neurosci, 2002. **100**(1-2): p. 66-76.
415. Arzeno, N.M., et al., *Sex differences in blood pressure control during 6 degrees head-down tilt bed rest.* Am J Physiol Heart Circ Physiol, 2013. **304**(8): p. H1114-23.
416. Bredella, M.A., *Sex Differences in Body Composition*, in *Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity*, F. Mauvais-Jarvis, Editor. 2017, Springer International Publishing: Cham. p. 9-27.
417. Arciero, P.J., M.I. Goran, and E.T. Poehlman, *Resting metabolic rate is lower in women than in men.* J Appl Physiol (1985), 1993. **75**(6): p. 2514-20.
418. Smith, S.M., et al., *Nutritional status assessment in semiclosed environments: ground-based and space flight studies in humans.* J Nutr, 2001. **131**(7): p. 2053-61.
419. Thomas, K.S. and H.J. McMann, *US Spacesuits*. 2007: Springer New York.
420. *NASA'S Development of Next Generation Spacesuits.* 2021.
421. McNulty, K.L., et al., *The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis.* Sports Med, 2020. **50**(10): p. 1813-1827.

422. Blagrove, R.C., G. Bruinvels, and C.R. Pedlar, *Variations in strength-related measures during the menstrual cycle in eumenorrheic women: A systematic review and meta-analysis.* J Sci Med Sport, 2020. **23**(12): p. 1220-1227.
423. Carmichael, M.A., et al., *The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review.* Int J Environ Res Public Health, 2021. **18**(4).
424. Elliott-Sale, K.J., et al., *The Effects of Oral Contraceptives on Exercise Performance in Women: A Systematic Review and Meta-analysis.* Sports Med, 2020. **50**(10): p. 1785-1812.
425. Taipale-Mikkonen, R.S., et al., *Influence of Menstrual Cycle or Hormonal Contraceptive Phase on Physiological Variables Monitored During Treadmill Testing.* Front Physiol, 2021. **12**: p. 761760.
426. Moore, A.D., P.A. Lynn, and A.H. Feiveson, *The First 10 Years of Aerobic Exercise Responses to Long-Duration ISS Flights.* Aerosp Med Hum Perform, 2015. **86**(12 Suppl): p. A78-A86.
427. Kashihara, H., et al., *Effects of mild supine exercise during 20 days bed rest on maximal oxygen uptake rate in young humans.* Acta Physiol Scand Suppl, 1994. **616**: p. 19-26.
428. Narici, M.V. and M.D. de Boer, *Disuse of the musculo-skeletal system in space and on earth.* Eur J Appl Physiol, 2011. **111**(3): p. 403-20.
429. Stein, T.P., *Weight, muscle and bone loss during space flight: another perspective.* Eur J Appl Physiol, 2013. **113**(9): p. 2171-81.
430. Deschenes, M.R., et al., *Gender influences neuromuscular adaptations to muscle unloading.* Eur J Appl Physiol, 2009. **105**(6): p. 889-97.
431. Deschenes, M.R., R.W. McCoy, and K.A. Mangis, *Factors relating to gender specificity of unloading-induced declines in strength.* Muscle Nerve, 2012. **46**(2): p. 210-7.
432. Yasuda, N., et al., *Sex-based differences in skeletal muscle function and morphology with short-term limb immobilization.* J Appl Physiol (1985), 2005. **99**(3): p. 1085-92.
433. MacLennan, R.J., et al., *The time course of neuromuscular impairment during short-term disuse in young women.* Physiol Rep, 2021. **9**(1): p. e14677.
434. Clark, B.C., et al., *Restoration of voluntary muscle strength after 3 weeks of cast immobilization is suppressed in women compared with men.* Arch Phys Med Rehabil, 2009. **90**(1): p. 178-80.
435. Vernikos, J., et al., *Effect of standing or walking on physiological changes induced by head down bed rest: implications for spaceflight.* Aviat Space Environ Med, 1996. **67**(11): p. 1069-79.
436. Wenger, H.A. and G.J. Bell, *The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness.* Sports Med, 1986. **3**(5): p. 346-56.
437. Greenleaf, J.E., et al., *Effect of leg exercise training on vascular volumes during 30 days of 6 degrees head-down bed rest.* J Appl Physiol (1985), 1992. **72**(5): p. 1887-94.
438. Convertino, V.A., et al., *Restoration of plasma volume after 16 days of head-down tilt induced by a single bout of maximal exercise.* Am J Physiol, 1996. **270**(1 Pt 2): p. R3-10.
439. Rittweger, J., et al., *Vertical jump performance after 90 days bed rest with and without flywheel resistive exercise, including a 180 days follow-up.* Eur J Appl Physiol, 2007. **100**(4): p. 427-36.
440. Trappe, S., et al., *Single muscle fiber function with concurrent exercise or nutrition countermeasures during 60 days of bed rest in women.* J Appl Physiol (1985), 2007. **103**(4): p. 1242-50.
441. Ploutz-Snyder, L.L., et al., *Integrated resistance and aerobic exercise protects fitness during bed rest.* Med Sci Sports Exerc, 2013: p. (in press).
442. Caiozzo, V.J., et al., *Artificial gravity as a countermeasure to microgravity: a pilot study examining the effects on knee extensor and plantar flexor muscle groups.* J Appl Physiol (1985), 2009. **107**(1): p. 39-46.

443. Zange, J., et al., *20-Hz whole body vibration training fails to counteract the decrease in leg muscle volume caused by 14 days of 6 degrees head down tilt bed rest.* Eur J Appl Physiol, 2009. **105**(2): p. 271-7.
444. Belavy, D.L., et al., *Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bed rest.* Osteoporos Int, 2011. **22**(5): p. 1581-91.
445. Dirks, M.L., et al., *Neuromuscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans.* Acta Physiol (Oxf), 2014. **210**(3): p. 628-41.
446. Birkhead, N.C., et al., *Cardiodynamic and Metabolic Effects of Prolonged Bed Rest with Daily Recumbent or Sitting Exercise and with Sitting Inactivity. Techn Docum Rep No. Amrl-Tdr-64-61.* AMRL TR, 1964: p. 1-28.
447. Lamb, L.E., R.L. Johnson, and P.M. Stevens, *Cardiovascular Deconditioning during Chair Rest.* Aerosp Med, 1964. **35**: p. 646-9.
448. Lamb, L.E., P.M. Stevens, and R.L. Johnson, *Hypokinesia Secondary to Chair Rest from 4 to 10 Days.* Aerosp Med, 1965. **36**: p. 755-63.
449. Iwasaki, K.I., et al., *Usefulness of daily +2Gz load as a countermeasure against physiological problems during weightlessness.* Acta Astronaut, 2001. **49**(3-10): p. 227-35.
450. Riviere, D., et al., *Physical fitness before and after one month head-down bedrest, with and without lower body negative pressure.* Physiologist, 1990. **33**(1 Suppl): p. S34-5.
451. Stenger, M.B., et al., *Artificial gravity training reduces bed rest-induced cardiovascular deconditioning.* Eur J Appl Physiol, 2012. **112**(2): p. 605-16.
452. Hughson, R.L., et al., *Reduced spontaneous baroreflex response slope during lower body negative pressure after 28 days of head-down bed rest.* J Appl Physiol (1985), 1994. **77**(1): p. 69-77.
453. Maillet, A., et al., *Cardiovascular and hormonal response during a 4-week head-down tilt with and without exercise and LBNP countermeasures.* J Gravit Physiol, 1996. **3**(1): p. 37-48.
454. Whalen, R., *Musculoskeletal adaptation to mechanical forces on Earth and in space.* Physiologist, 1993. **36**(1 Suppl): p. S127-30.
455. Moore, A.D., et al., *Aerobic deconditioning and recovery following long duration flight onboard the International Space Station.* Med Sci Sports Exerc, 2003. **35**: p. 263.
456. Smith, S.M., et al., *Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes.* Am J Physiol, 1999. **277**(1 Pt 2): p. R1-10.
457. Meck, J.V., et al., *Marked exacerbation of orthostatic intolerance after long- vs. short-duration spaceflight in veteran astronauts.* Psychosom Med, 2001. **63**(6): p. 865-73.
458. Lee, S.M.C., et al., *Concentric strength and endurance after long duration spaceflight.* Med Sci Sports Exerc, 2000. **32**: p. S363.
459. Murthy, G., et al., *Exercise against lower body negative pressure as a countermeasure for cardiovascular and musculoskeletal deconditioning.* Acta Astronaut, 1994. **33**: p. 89-96.
460. Murthy, G., et al., *Supine exercise during lower body negative pressure effectively simulates upright exercise in normal gravity.* J Appl Physiol (1985), 1994. **76**(6): p. 2742-8.
461. Watenpaugh, D.E., et al., *Lower body negative pressure exercise during bed rest maintains orthostatic tolerance.* FASEB J, 1994. **8**: p. A261.
462. Cao, P., et al., *Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest.* J Appl Physiol, 2005. **99**(1): p. 39-44.
463. Schneider, S.F., et al., *Twins Bed Rest Project: Gender differences in loss of leg strength and endurance during bed rest.* FASEB J., 2006. **20**(5): p. A1252-b.

464. Lee, S.M., et al., *WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women.* J Appl Physiol (1985), 2014. **116**(6): p. 654-67.
465. Dillon, E.L., et al., *Efficacy of Testosterone plus NASA Exercise Countermeasures during Head-Down Bed Rest.* Med Sci Sports Exerc, 2018. **50**(9): p. 1929-1939.
466. Baldwin, K.M., et al., *Alterations in muscle mass and contractile phenotype in response to unloading models: role of transcriptional/pretranslational mechanisms.* Front Physiol, 2013. **4**: p. 284.
467. Brooks, N.E. and K.H. Myburgh, *Skeletal muscle wasting with disuse atrophy is multi-dimensional: the response and interaction of myonuclei, satellite cells and signaling pathways.* Front Physiol, 2014. **5**: p. 99.
468. Stabley, J.N., et al., *Chronic skeletal unloading of the rat femur: mechanisms and functional consequences of vascular remodeling.* Bone, 2013. **57**(2): p. 355-60.
469. Sindler, A.L., et al., *Effects of ageing and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles.* J Physiol, 2009. **587**(Pt 15): p. 3885-97.
470. Thomason, D.B. and F.W. Booth, *Atrophy of the soleus muscle by hindlimb unweighting.* J Appl Physiol (1985), 1990. **68**(1): p. 1-12.
471. Thomason, D.B., et al., *Time course of soleus muscle myosin expression during hindlimb suspension and recovery.* J Appl Physiol (1985), 1987. **63**(1): p. 130-7.
472. Thomason, D.B., et al., *Altered actin and myosin expression in muscle during exposure to microgravity.* J Appl Physiol (1985), 1992. **73**(2 Suppl): p. 90S-93S.
473. Recktenwald, M.R., et al., *Effects of spaceflight on rhesus quadrupedal locomotion after return to 1G.* J Neurophysiol, 1999. **81**(5): p. 2451-63.
474. Alford, E.K., et al., *Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension.* Exp Neurol, 1987. **96**(3): p. 635-49.
475. Riley, D.A., et al., *In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats.* J Appl Physiol (1985), 1996. **81**(1): p. 133-44.
476. Roy, R.R., K.M. Baldwin, and V.R. Edgerton, *Response of the neuromuscular unit to spaceflight: what has been learned from the rat model.* Exercise and Sport Sciences Reviews, 1996. **24**: p. 399-425.
477. Baldwin, K.M., *Effect of spaceflight on the functional, biochemical, and metabolic properties of skeletal muscle.* Med Sci Sports Exerc, 1996. **28**(8): p. 983-7.
478. Baldwin, K.M., *Effects of altered loading states on muscle plasticity: what have we learned from rodents?* Medicine and Science in Sports and Exercise, 1996. **28**(10 Suppl): p. S101-6.
479. Baldwin, K.M., et al., *Effects of zero gravity on myofibril content and isomyosin distribution in rodent skeletal muscle.* FASEB J, 1990. **4**(1): p. 79-83.
480. Baldwin, K.M., R.E. Herrick, and S.A. McCue, *Substrate oxidation capacity in rodent skeletal muscle: effects of exposure to zero gravity.* J Appl Physiol (1985), 1993. **75**(6): p. 2466-70.
481. Dupont, E., et al., *Electrostimulation during hindlimb unloading modulates PI3K-AKT downstream targets without preventing soleus atrophy and restores slow phenotype through ERK.* Am J Physiol Regul Integr Comp Physiol, 2011. **300**(2): p. R408-17.
482. McDonald, K.S., M.D. Delp, and R.H. Fitts, *Fatigability and blood flow in the rat gastrocnemius-plantaris-soleus after hindlimb suspension.* J Appl Physiol (1985), 1992. **73**(3): p. 1135-40.
483. McDonald, K.S. and R.H. Fitts, *Effect of hindlimb unweighting on single soleus fiber maximal shortening velocity and ATPase activity.* J Appl Physiol (1985), 1993. **74**(6): p. 2949-57.
484. Caiozzo, V.J., et al., *Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle.* J Appl Physiol (1985), 1994. **76**(4): p. 1764-73.

485. Caiozzo, V.J., et al., *Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle.* J Appl Physiol (1985), 1996. **81**(1): p. 123-32.
486. Ilyina-Kakueva, E.I., V.V. Portugalov, and N.P. Krivenkova, *Space flight effects on the skeletal muscles of rats.* Aviat Space Environ Med, 1976. **47**(7): p. 700-3.
487. Martin, T.P., V.R. Edgerton, and R.E. Grindeland, *Influence of spaceflight on rat skeletal muscle.* J Appl Physiol (1985), 1988. **65**(5): p. 2318-25.
488. Ohira, Y., et al., *Rat soleus muscle fiber responses to 14 days of spaceflight and hindlimb suspension.* J Appl Physiol (1985), 1992. **73**(2 Suppl): p. 51S-57S.
489. Fitts, R.H., et al., *Models of disuse: a comparison of hindlimb suspension and immobilization.* J Appl Physiol (1985), 1986. **60**(6): p. 1946-53.
490. Roy, R.R., K.M. Baldwin, and V.R. Edgerton, *The plasticity of skeletal muscle: effects of neuromuscular activity.* Exerc Sport Sci Rev, 1991. **19**: p. 269-312.
491. Gardetto, P.R., J.M. Schluter, and R.H. Fitts, *Contractile function of single muscle fibers after hindlimb suspension.* J Appl Physiol (1985), 1989. **66**(6): p. 2739-49.
492. Haddad, F., et al., *Myosin heavy chain expression in rodent skeletal muscle: effects of exposure to zero gravity.* J Appl Physiol (1985), 1993. **75**(6): p. 2471-7.
493. Musacchia, X.J., et al., *Skeletal muscle atrophy in response to 14 days of weightlessness: vastus medialis.* J Appl Physiol (1985), 1992. **73**(2 Suppl): p. 44S-50S.
494. Diffee, G.M., et al., *Contractile and biochemical properties of rat soleus and plantaris after hindlimb suspension.* Am J Physiol, 1991. **260**(3 Pt 1): p. C528-34.
495. Giger, J.M., et al., *Rapid muscle atrophy response to unloading: pretranslational processes involving MHC and actin.* J Appl Physiol (1985), 2009. **107**(4): p. 1204-12.
496. Pandorf, C.E., et al., *Dynamics of myosin heavy chain gene regulation in slow skeletal muscle: role of natural antisense RNA.* J Biol Chem, 2006. **281**(50): p. 38330-42.
497. Van Gammeren, D., et al., *The IkappaB kinases IKKalpha and IKKbeta are necessary and sufficient for skeletal muscle atrophy.* FASEB J, 2009. **23**(2): p. 362-70.
498. Schulte, L.M., J. Navarro, and S.C. Kandarian, *Regulation of sarcoplasmic reticulum calcium pump gene expression by hindlimb unweighting*.
499. Chi, M.M., et al., *Effects of microgravity and tail suspension on enzymes of individual soleus and tibialis anterior fibers.* J Appl Physiol (1985), 1992. **73**(2 Suppl): p. 66S-73S.
500. Goodpaster, B.H. and L.M. Sparks, *Metabolic Flexibility in Health and Disease.* Cell Metab, 2017. **25**(5): p. 1027-1036.
501. Stevens, L., Y. Mounier, and X. Holy, *Functional adaptation of different rat skeletal muscles to weightlessness.* Am J Physiol, 1993. **264**(4 Pt 2): p. R770-6.
502. McDonald, K.S., M.D. Delp, and R.H. Fitts, *Effect of hindlimb unweighting on tissue blood flow in the rat.* J Appl Physiol (1985), 1992. **72**(6): p. 2210-8.
503. Chegwidden, W.R. and D.C. Watts, *Kinetic studies and effects of anions on creatine phosphokinase from skeletal muscle of rhesus monkey (Macaca mulatta).* Biochim Biophys Acta, 1975. **410**(1): p. 99-114.
504. Riley, D.A., et al., *Review of spaceflight and hindlimb suspension unloading induced sarcomere damage and repair.* Basic Appl Myol, 1995. **5**(2): p. 139-45.
505. Krippendorf, B.B. and D.A. Riley, *Distinguishing unloading- versus reloading-induced changes in rat soleus muscle.* Muscle Nerve, 1993. **16**(1): p. 99-108.
506. Riley, D.A., *Review of primary spaceflight-induced and secondary reloading-induced changes in slow antigravity muscles of rats.* Adv Space Res, 1998. **21**(8-9): p. 1073-5.
507. Giger, J.M., et al., *Effect of unloading on type I myosin heavy chain gene regulation in rat soleus muscle.* J Appl Physiol (1985), 2005. **98**(4): p. 1185-94.
508. Adams, G.R., et al., *Combined isometric, concentric, and eccentric resistance exercise prevents unloading-induced muscle atrophy in rats.* J Appl Physiol (1985), 2007. **103**(5): p. 1644-54.

509. Haddad, F., et al., *Isometric resistance exercise fails to counteract skeletal muscle atrophy processes during the initial stages of unloading.* J Appl Physiol (1985), 2006. **100**(2): p. 433-41.
510. Fielitz, J., et al., *Myosin accumulation and striated muscle myopathy result from the loss of muscle RING finger 1 and 3.* J Clin Invest, 2007. **117**(9): p. 2486-95.
511. Fitts, R.H., et al., *Velocity, force, power, and Ca2+ sensitivity of fast and slow monkey skeletal muscle fibers.* J Appl Physiol (1985), 1998. **84**(5): p. 1776-87.
512. Fitts, R.H., et al., *Spaceflight effects on single skeletal muscle fiber function in the rhesus monkey.* Am J Physiol Regul Integr Comp Physiol, 2000. **279**(5): p. R1546-57.
513. Kelleher, A.R., et al., *Changes in REDD1, REDD2, and atrogene mRNA expression are prevented in skeletal muscle fixed in a stretched position during hindlimb immobilization.* Physiol Rep, 2014. **2**(2): p. e00246.
514. Fitts, R.H., D.R. Riley, and J.J. Widrick, *Physiology of a Microgravity Environment Invited Review: Microgravity and skeletal muscle.* J Appl Physiol, 2000. **89**(2): p. 823-839.
515. Fitts, R.H., et al., *Prolonged space flight-induced alterations in the structure and function of human skeletal muscle fibres.* J Physiol, 2010. **588**(18): p. 3567-3592.
516. Roy, R.R., et al., *Recruitment of the Rhesus soleus and medial gastrocnemius before, during and after spaceflight.* J Gravit Physiol, 1996. **3**(1): p. 11-5.
517. Allen, D.L., et al., *Effects of spaceflight on murine skeletal muscle gene expression.* J Appl Physiol (1985), 2009. **106**(2): p. 582-95.
518. Haddad, F., et al., *Role of antisense RNA in coordinating cardiac myosin heavy chain gene switching.* J Biol Chem, 2003. **278**(39): p. 37132-8.
519. Mahdavi, V., A.P. Chambers, and B. Nadal-Ginard, *Cardiac alpha- and beta-myosin heavy chain genes are organized in tandem.* Proc Natl Acad Sci U S A, 1984. **81**(9): p. 2626-30.
520. Haddad, F., et al., *Cardiac myosin heavy chain gene regulation by thyroid hormone involves altered histone modifications.* Am J Physiol Heart Circ Physiol, 2010. **299**(6): p. H1968-80.
521. Haddad, F., et al., *Intergenic transcription and developmental regulation of cardiac myosin heavy chain genes.* Am J Physiol Heart Circ Physiol, 2008. **294**(1): p. H29-40.
522. Haddad, F., et al., *Regulation of antisense RNA expression during cardiac MHC gene switching in response to pressure overload.* Am J Physiol Heart Circ Physiol, 2006. **290**(6): p. H2351-61.
523. Pandorf, C.E., et al., *Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading.* Am J Physiol, 2009. **297**(1): p. C6-C16.
524. Jackman, R.W., C.L. Wu, and S.C. Kandarian, *The ChIP-seq-defined networks of Bcl-3 gene binding support its required role in skeletal muscle atrophy.* PLoS One, 2012. **7**(12): p. e51478.
525. Maki, T., et al., *Branched-chain amino acids reduce hindlimb suspension-induced muscle atrophy and protein levels of atrogin-1 and MuRF1 in rats.* Nutr Res, 2012. **32**(9): p. 676-83.
526. Derbre, F., et al., *Inhibition of xanthine oxidase by allopurinol prevents skeletal muscle atrophy: role of p38 MAPKinase and E3 ubiquitin ligases.* PLoS One, 2012. **7**(10): p. e46668.
527. Fujino, H., et al., *Protective effects of exercise preconditioning on hindlimb unloading-induced atrophy of rat soleus muscle.* Acta Physiol (Oxf), 2009. **197**(1): p. 65-74.
528. Bruusgaard, J.C., et al., *No change in myonuclear number during muscle unloading and reloading.* J Appl Physiol (1985), 2012. **113**(2): p. 290-6.
529. Allen, D.L., et al., *Apoptosis: a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting.* Am J Physiol, 1997. **273**(2 Pt 1): p. C579-87.

530. Bortoloso, E., et al., *Homer 2 antagonizes protein degradation in slow-twitch skeletal muscles.* Am J Physiol Cell Physiol, 2013. **304**(1): p. C68-77.
531. Kelleher, A.R., et al., *The mTORC1 signaling repressors REDD1/2 are rapidly induced and activation of p70S6K1 by leucine is defective in skeletal muscle of an immobilized rat hindlimb.* Am J Physiol Endocrinol Metab, 2013. **304**(2): p. E229-36.
532. Nakao, R., et al., *Ubiquitin ligase Cbl-b is a negative regulator for insulin-like growth factor 1 signaling during muscle atrophy caused by unloading.* Mol Cell Biol, 2009. **29**(17): p. 4798-811.
533. Kazi, A.A., et al., *Deptor knockdown enhances mTOR Activity and protein synthesis in myocytes and ameliorates disuse muscle atrophy.* Mol Med, 2011. **17**(9-10): p. 925-36.
534. Shtifman, A., et al., *Divergent modification of low-dose (5)(6)Fe-particle and proton radiation on skeletal muscle.* Radiat Res, 2013. **180**(5): p. 455-64.
535. Pogatsa, G., *Bilinear correlation between tissue water content and diastolic stiffness of the ventricular myocardium.* Experientia, 1980. **36**(12): p. 1402-3.
536. Griffin D, M.L., *The NASA Digital Astronaut Project.* 2012.
537. Waligora, J.M. and K.V. Kumar, *Energy utilization rates during shuttle extravehicular activities.* Acta Astronaut, 1995. **36**(8-12): p. 595-9.
538. Vos, J.R., Gernhardt, Michael L., Lee, Lesley, *The Walkback Test: A Study to Evaluate Suit and Life Support System Performance Requirements for a 10 Kilometer Traverse in a Planetary Suit.* 2013.
539. Sawin, C.F., Taylor, Gerald R., Smith, Wanda L., Brown, J. Travis, *Extended Duration Orbiter Medical Project.* NTRS - NASA Technical Reports Server, 1999.
540. Frenkel D, B.C., Huffman RK, Quiocho LJ, Lostroscio KH, *Human Stability While Exercising on a VIS Device in Zero Gravity.* 2019.
541. Holt, J.A., et al., *WISE 2005: Aerobic and resistive countermeasures prevent paraspinal muscle deconditioning during 60-day bed rest in women.* J Appl Physiol (1985), 2016. **120**(10): p. 1215-22.
542. Meuche, S.S., SM; Lee, SMC; Macias, BR; Smith, SM; Watenpaugh, DE; Hargens, AR, *WISE 2005: LBNP Exercise and Flywheel Resistive Exercise as an Effective Countermeasure Combination.* 2013.
543. Scheuring, R.A., Jones, J.A., Polk, J.D., Gillis, D.B, Schmid, J., Duncan, J.M., Davis, J.R., Novak, J.D., *The Apollo Medical Operations Project: Recommendations to Improve Crew Health and Performance for Future Exploration Missions and Lunar Surface Operations*. Vol. TM-2007-214755. 2007, Houston: NASA/Johnson Space Center.
544. *NASA'S Management of the Gateway Program for Artemis Missions.* 2020.
545. Hughson, R.L., et al., *Increased postflight carotid artery stiffness and inflight insulin resistance resulting from 6-mo spaceflight in male and female astronauts.* Am J Physiol Heart Circ Physiol, 2016. **310**(5): p. H628-38.