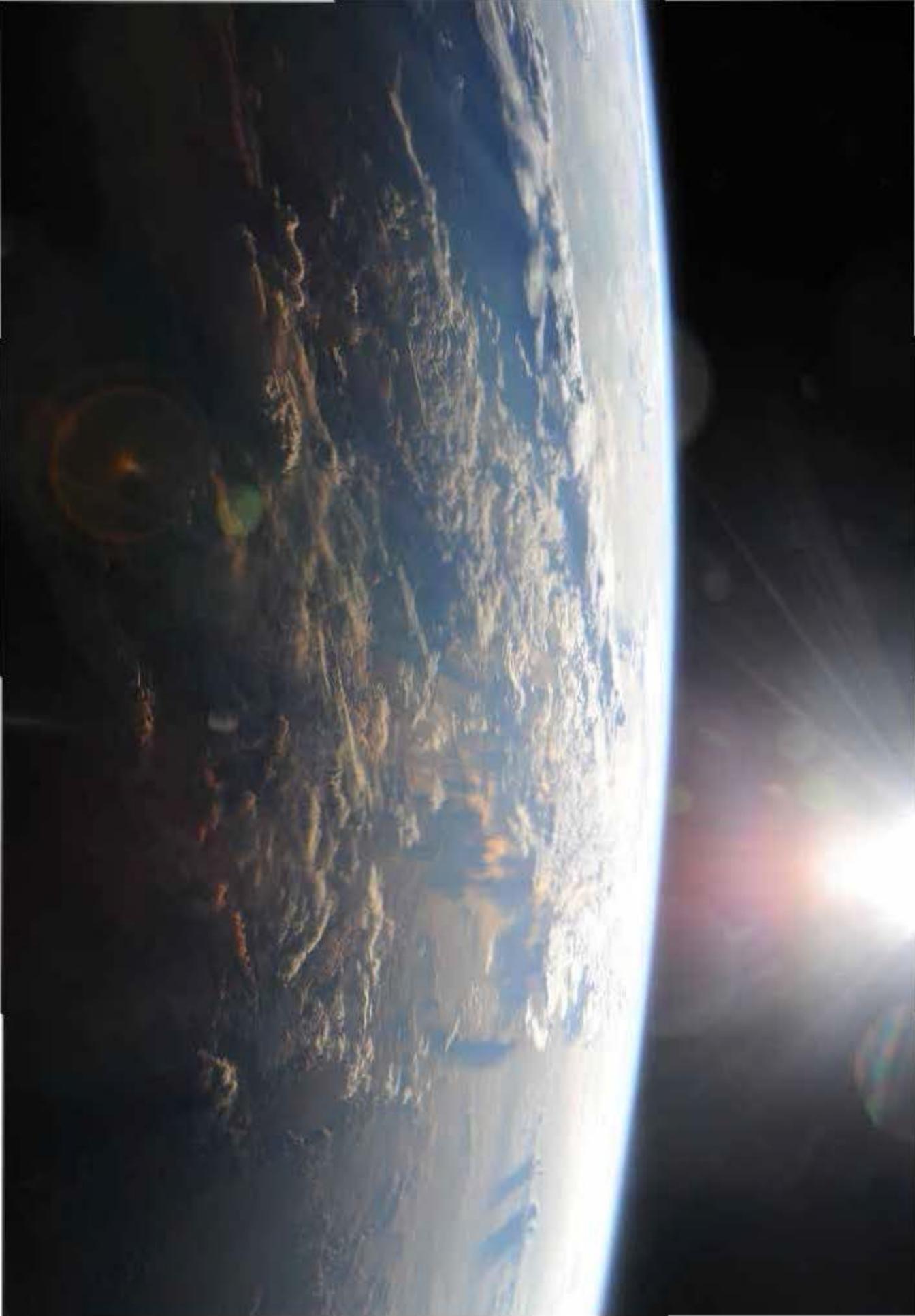National Aeronautics and
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Human Adaptation to Spaceflight: The Role of Nutrition

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HUMAN ADAPTATION TO SPACE FLIGHT: THE ROLE OF NUTRITION

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Preface

Our first effort at compiling a book took place in 2009. The product of that effort is available for (free) download through the link http://go.nasa.gov/QS1KW1. That initial effort came from a push within the NASA Human Research Program to have each of the disciplines or "Elements" comprising the Program prepare an "evidence report" describing, as thoroughly as possible, "everything" known about the discipline (in our case, Space Nutrition), including gaps in our knowledge that needed to be filled before we could take the next steps of exploration. Details were provided to allow definition of space flight requirements, and to that end, the text of the Space Nutrition book included basics of nutrient metabolism and requirements on Earth, and much of the available data related to space flight. Because, to a degree, the format was thrust upon us, and the timeline was short, the 2009 book reads a little like an encyclopedia.

The current volume is not a second edition of that first book, but rather an evolution. It is our hope that this book reflects a (brief) review of the history of and current state of knowledge about the role of nutrition in human space flight. We have attempted to organize this from a more physiological point of view, and to highlight systems, and the nutrients that support them, rather than the other way around.

The other main difference between the 2 books is that a tremendous amount of work has been completed and published in the past 6 years, including many reports of ground-based studies and International Space Station (ISS) flight research findings. New risks to human health have been identified, including one related to vision changes in astronauts on ISS. We detail herein data suggesting a tie-in of the folate- and vitamin B_{12}-dependent 1-carbon metabolism pathway with these changes. Recent publications have documented the effects of good nutrition and heavy resistance exercise on bone metabolism during space flight. After more than a half century of human space flight, this is the first evidence of the ability to mitigate the loss of bone mineral density in astronauts on long-duration missions. Although more work remains to be done, any progress is incredibly exciting.

We hope we have captured in this book the state of the field of study of the role of human nutrition in space flight, along with the work leading up to this state, and some guideposts for work remaining to be done and gaps that need to be filled.

1 Introduction

The importance of nutrition in exploration has been documented repeatedly throughout history, on voyages across oceans, on expeditions across polar ice, and on treks across unexplored continents. Scurvy is a prime example of exploration-induced nutritional discovery. While most people understand that scurvy is associated with vitamin C deficiency, and that this was an issue for sailors centuries ago, few realize the magnitude of this problem. If one examines the roughly 400-year period between Christopher Columbus' voyage in 1492 and the invention of the steam engine, scurvy resulted in the deaths of more sailors than all other causes of death combined (1). Ships that sailed with a crew of hundreds often returned with tens. It is estimated that more than 2 million sailors perished from scurvy during this time (1). A key difference between these difficult journeys and future space exploration is that astronauts are not likely to find food along the way. Thus, understanding the nutritional requirements of space travelers and the role of nutrition in human adaptation to microgravity are as critical to crew safety and mission success as any of the mechanical systems of the spacecraft itself.

There are many facets to nutrition and health on Earth. Space flight introduces further complications, and many gaps remain in our knowledge of the relationships between nutrition and health in space that need to be filled before we can safely embark on "exploration" missions, that is, missions beyond low Earth orbit. At the surface of these unknowns is the need to understand and define basic nutrient requirements during extended stays in microgravity. Beyond this lies the need to characterize the role of nutrition in the adaptation of physiological systems to microgravity, and/or the impact of these changes on nutrition. Additionally, environmental impacts (including radiation, and spacecraft and spacesuit atmospheres) can alter nutritional status and nutritional requirements of space flight. For surface missions (on, for example, the moon and Mars), partial gravity may complicate the situation further. Finally, many potential targets for nutritional countermeasures exist, where modified dietary intake may help to counteract or mitigate some of the negative effects of space flight on the human body.

In 2009, at the urging of NASA's Human Research Program, the authors published a book (2) with the aim of reviewing the existing knowledge and history of human nutrition for space flight, with a key goal of identifying gaps in the knowledge base required to provide confidence that the risk of an inadequate food system or inadequate nutrition to support humans on expeditions to the moon and Mars is as low as possible. A brief history of space programs and space food systems was also included. This volume is available for free download through the link http://go.nasa.gov/QS1KW1.

What we have attempted to provide here is not a second edition, but rather an updated overview of space nutrition from a more physiological perspective. Its division into chapters is based on physiological systems (eg, metabolism, muscle, bone), and highlighted in each chapter are the nutrients that are particularly associated with that system. We present data from ground-based analog studies, conducted in laboratories on Earth by exposing human subjects to one or more environmental conditions similar to those produced by space flight. While bed rest may be the most common analog of space flight, recent nutrition research from Antarctica and undersea habitats has helped

expand our understanding of nutritional changes during space flight simulations. We will also review space flight research, including data from now-historical flights on the Space Shuttle, as well as the Russian space station Mir and earlier space programs such as Apollo and Skylab missions. In recent years, the International Space Station (ISS) has provided (and continues to provide) a wealth of nutrition findings from flights of 4 to 6 months, and these will be reviewed in detail. We also present a brief overview of the techniques used for conducting this research on orbit.

Key areas of nutrition concerns are primarily nutrition- or nutrient-related changes, including loss of body mass and depletion of body nutrient stores because of inadequate food supply, inadequate food intake, increased metabolism, and/or irreversible loss or degradation of nutrients. Physiological changes that involve nutrition (to some degree) include bone and muscle loss, cardiovascular degradation, impairment of immune function, and neurovestibular changes. Vision changes have recently been identified in ISS crewmembers, and are a major (if not the major) health concern for long-term space missions (3). Environmental issues, including radiation exposure and cabin environment (O_2/CO_2, temperature, and humidity) can have profound effects on nutrition, and/or may provide areas where nutrients may serve as countermeasures. When crewmembers are outside the spacecraft (that is, on spacewalks, or extravehicular activity), the spacesuit becomes a spacecraft as well, with associated concerns (high oxygen exposure, limited water availability, inability to eat for up to 8 to 10 hours at a time while in the suit). Drug-nutrient interactions are of concern on Earth, and take on added importance during space flight. We will review all of these areas in general, and in detail with respect to the role of nutrition and specific nutrients, highlighting existing knowledge as well as gaps where additional research is needed.

Conducting Nutrition Research on ISS

Blood Collection

The first crew of ISS took up residence there in November 2000, but the turning point for nutrition research on ISS came in 2006, when the capability for collection, processing, and frozen storage of blood and urine samples was brought to orbit. Crewmembers are trained in procedures and use of the equipment required, and depending on the planned crew complement and schedules, many are trained for autophlebotomy, that is, drawing their own blood. In October 2006, early in the Expedition 14 mission, Mike Lopez-Alegria collected the first blood samples to be drawn on ISS and captured the image (Figure 1). After they are collected, samples are allowed to clot, and are then centrifuged (Figure 2).

Figure 1. Mike Lopez-Alegria collects the first blood sample on the International Space Station on October 5, 2006, having inserted the needle himself. The collection tubes can be seen (one in hand, the others in elastic bands on his belt assembly), along with a sharps container and detailed procedures (both Velcroed to the wall). He had set the camera to automatically take pictures periodically, snapping this one in the process.

Figure 2. The refrigerated centrifuge on ISS.

Urine Collection

Urine is collected by using urine collection devices, UCDs (Figure 3; Figure 4). A UCD is essentially a bag with a one-way valve on one end, allowing urine entry while voiding, and a port at the other end to withdraw syringe samples. Each void, typically over a 24-hour period, is collected in this manner, and 2 or 3 syringes are used per void to withdraw about 6 to 7 mL of urine. Before flight, a small amount of lithium chloride solution is added to the bags, and after the void is complete, the UCD is kneaded to facilitate mixing of the LiCl with the void. After the samples are returned to Earth, the lithium concentration is determined, to allow back-calculation of the urine void volume so that 24-hour pools may be created from the individual voids.

Figure 3. Left: Urine Collection Device (UCD), shown here with a syringe on the collection port, and a female adapter. Right: a UCD with a male adapter shown floating on ISS.

After the syringes are collected, the UCD is placed in a ziplock bag to provide another layer of containment, and the ziplock is placed in a urine containment bag (UCB, Figure 5). Typically, after the day's collections are complete, the UCBs are stowed for eventual disposal with other ISS trash. Most trash is discarded in departing cargo vehicles that are not designed for reentry (that is, they burn up coming back through the atmosphere). Before the Space Shuttle was retired in 2011, some trash was brought home on Shuttle vehicles.

Figure 4. Sunita Williams shown here with a UCD, contained in a ziplock bag to provide another layer of containment.

Biological Sample Stowage and Return

The other key piece of hardware launched to ISS in 2006 was the freezer, the minus-eighty (degrees) laboratory freezer for ISS, or MELFI. There are currently 3 MELFIs on ISS, to allow increased stowage volume for intervals between returns to Earth. Each MELFI has 4 dewars (double-walled containers). On the outside of the MELFI, the dewars are covered (Figure 6 shows a crewmember opening the lid of one of the dewars). Each dewar has 4 trays, which are pulled out to store samples (Figures 7-9). The MELFIs are primarily designed for ultracold storage (temperatures lower than -80°C), and typically maintain temperatures close to -96°C. They are capable of refrigerated storage as well; typically one of the 4 dewars will be at refrigerator temperature, while the others will be at -96°.

Figure 5. Urine Containment Bag, used for holding discarded UCDs until they can be disposed of along with other trash from ISS.

Figure 6. External view of a MELFI, showing the covers for the 4 dewars.

Figure 7. External view of a MELFI, showing the interior of one of the dewars, with 1 tray partially removed. The 2 urine syringes to be placed in the freezer can be seen Velcroed to the wall.

Whenever possible, samples are returned to Earth. The Space Shuttle was the only ride home for frozen blood and urine samples before its retirement in 2011. On the first few return flights, samples were transferred from the MELFI to Double Cold Bags (Figure 9), which

Figure 8. Closer view of the MELFI tray, with compartment open for sample insertion. The mesh bags are designed to contain the samples from a given blood collection session. Blood samples are shown in this image.

Figure 9. Crewmember Greg Chamitoff (left) can be seen with the MELFI tray under his right hand, performing sample transfers from the MELFI into the Double Cold Bag (Sandy Magnus has her hand inside the DCB in this image).

included specially designed ice packs to maintain samples in a frozen state (specifically, at a lower temperature than -30°C) from the time when samples were taken out of the MELFI through the time when they were received at the landing site, usually 2 to 3 hours after landing. The ice packs would maintain required temperatures for more than 125 hours. This duration was required because the Space Shuttle usually stayed on orbit for 1 to 2 days after undocking from ISS, and could potentially be held up from landing because of weather issues at landing sites for another 1 to 2 days. On many sample return flights, in addition to the Double Cold Bags, a powered -96°C freezer is also flown to increase the volume of returning samples.

Since the retirement of the Space Shuttle, the only space cargo vehicles that have been able to return payloads are the SpaceX "Dragon" capsules. Other cargo vehicles exist, but they are only designed to bring supplies and equipment to ISS, not to return them to Earth. Thus, these other vehicles will burn up in the atmosphere on return, by design. The SpaceX capsules splash down in the ocean off the coast of California.

Food Intake Monitoring

Methods of recording food intake have evolved over the course of human space flight. On Skylab missions, which included metabolic balance studies, crewmembers called down daily to mission control and reported any off-nominal intake for the day. When it is required for specific research protocols, detailed dietary intake data has been obtained through written food intake logs or even with barcode scanning. Barcode scanning often seems an attractive alternative, but it comes with its own set of issues when used during space flight. Not all space foods have barcode labels on them, or for many commercially packaged items, the label is not in the database on the reader, and thus item information won't display on the screen. Additionally, many of the space foods do not have flat surfaces on their packaging, and wrinkles in the label can be difficult for the barcode reader to detect.

It was determined during the Shuttle-Mir program that for long-duration missions it would be valuable for flight surgeons to have a means of having the crews easily, and quickly, report typical dietary intake. To this end, a Food Frequency Questionnaire (FFQ) (Figure 10; Figure 11) was designed with the goal of having crews report typical intake once per week, with a computerized questionnaire that would take 5 to 15 minutes to complete. The FFQ was designed to report 6 key nutrients: energy, fluid, protein, calcium, sodium, and iron. Later a seventh nutrient, potassium, was included as well. The development, testing, and data from the FFQ have been reported (4-6). Although some crewmembers expressed concern over the accuracy of a 7-day recall questionnaire, the FFQ had
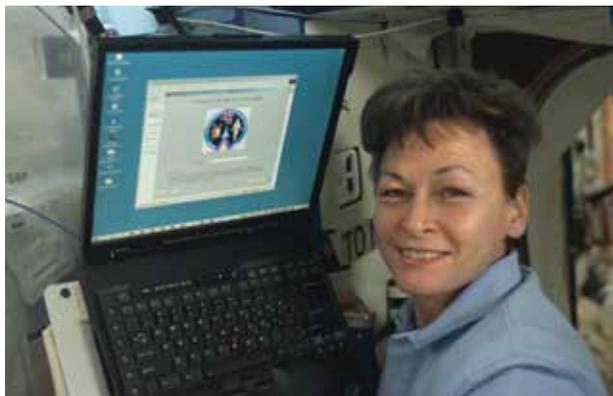

Figure 10. Peggy Whitson with the opening screen of the Food Frequency Questionnaire. After a crewmember enters name or initials, the second screen opens with a list of food categories, under each of which is a list of food items grouped together according to nutrient content.
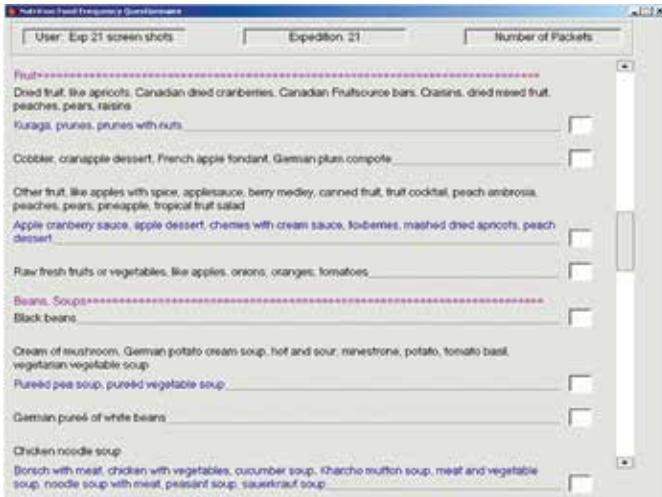
Figure 11. A screen shot from the Food Frequency Questionnaire. Available foods are grouped according to key nutrient content. Shown here are 2 groups: "Fruit" and "Beans, Soups." The foods provided by the European Space Agency, the Canadian Space Agency, the Japanese Aerospace Exploration Agency, and NASA are shown in black font, and foods provided by Russia are shown in blue.

several things in its favor: the ISS food system is rather limited, the portion sizes are known, and the available foods repeat with a high rate of frequency (with a menu cycle of 8 to 11 d).

While this text was being written (2013-2014) an iPad App was being developed for potential flight to ISS. Dubbed the ISS Food Intake Tracker, it has the purpose of enabling crews to more easily and accurately collect complete dietary intake data, in support of both medical operations and research experiment purposes.

Body Mass

Determining body mass is one of the most basic overall measures of health, as noted every time one visits a physician's office on Earth. Determination of "weight" in weightlessness, however, presents some unique challenges, as described in a recent report (7). There are two devices on ISS: a Body Mass Measuring Device, which uses spring oscillation, and a Space Linear Acceleration Mass Measuring Device, which uses the physics of the equation: force = mass x acceleration. Images of the two devices available on ISS are shown in Figure 12.

Figure 12. Karen Nyberg (left), Expedition 36 Flight Engineer, using the Space Linear Acceleration Mass Measurement Device. Tom Marshburn (right), Expedition 34 Flight Engineer, using the Body Mass Measurement Device. Photo credits: NASA.

2 Energy and Fuel Metabolism

Adequate energy intake is perhaps the single most important aspect of astronaut nutrition. This is not only because energy in and of itself is more important than other nutritional factors, but also because if enough food is consumed to meet energy needs, then generally other nutrients will also be consumed in reasonable amounts. There are many facets to maintaining eucaloric intake during space flight, including energy requirements; physiological changes in taste and satiety; scheduling issues of allotting time for meal preparation, consumption, and cleanup; food quality; and even food availability.

Little research has been done on differences in fuel components (protein, carbohydrate, fat) during space flight, or on cofactors (eg, vitamins) of energy utilization. We review these here, highlighting what has been done and potential areas of future research.

Energy Expenditure and Requirements

Energy expenditure is often hypothesized to be lower during flight than on the ground, because of the presumed relative hypokinesia in space (8). An early example of this is that lower energy expenditure was observed during extravehicular activity (EVA) on the lunar surface than during similar activities at 1*g* (9). This was determined through indirect calorimetry in the space suit. However, Space Shuttle crewmembers during EVA did not have any change in energy expenditure relative to before flight (10).

Studies of total energy expenditure of Shuttle astronauts documented that in-flight energy expenditure was unchanged from preflight levels (11), or in cases where these crews performed intensive exercise during the mission, energy expenditure during flight was higher than before flight (12). For these studies, the doubly-labeled water (water enriched with deuterium and ^{18}O) technique was used to determine oxygen consumption (13). The benefits of this technique are that it is noninvasive and it takes into account the energy cost of all activities over a period of several days. The drawback of the method is that information about the individual components of total energy expenditure (TEE), such as resting, sleep, and exercise, is not available. The range of differences between preflight and in-flight TEE makes it important to have information about the components of TEE. Although it is assumed that moving the body mass around the cabin requires less expenditure of energy during weightlessness than at 1*g*, other metabolic activities, such as maintaining resting metabolic rate and responding to stress, may require increased energy expenditure during weightlessness.

In ground-based bed rest studies, an analog of space flight, resting energy expenditure did not change, but TEE was less during bed rest than before bed rest (14). Because TEE during flight is unchanged (11) or increased (12) from preflight levels, the lower TEE during bed rest may indicate that bed rest is not an appropriate model for studies of energy metabolism during flight. One possible explanation for this difference between bed rest and space flight is the lack of a metabolic response to stress during bed rest (15). Attempts have been made to improve the utility of bed rest studies by administering a metabolic stressor (such as triiodothyronine or cortisol) to provide

a better ground-based model than bed rest alone for the metabolic effects of space flight on energy and fuel metabolism (16).

Energy requirements are typically estimated using standard equations, including the World Health Organization (WHO) (17) and Dietary Reference Intake (DRI) (18), using a "moderately active" or "active" adjustment for activity level for these 2 equations, respectively. The DRI equation includes the effects of age, sex, weight, and height in estimating energy requirements. While it would be more accurate to determine actual resting energy expenditure before flight for each astronaut, this testing has not been possible except in cases where these data were required for specific experiments.

The aforementioned studies of energy expenditure on Space Shuttle missions were performed on flights of 10 to 14 days' duration. The objective of a European Space Agency (ESA)-sponsored experiment initiated in 2012 is to determine energy expenditure during 6-month missions to the International Space Station. These data will help us understand whether any adaptation effect occurs on these longer missions, and thus may be important in estimating energy requirements for exploration missions (missions beyond low Earth orbit).

Energy Intake

Historically, inadequate energy intake and subsequent body mass loss have been considered hallmarks of space flight, and have occurred on many missions and programs (2, 4, 6, 11, 19-27). From Apollo through the Shuttle program, crewmember dietary intakes during flight averaged about 70% of predicted requirements (6), and ISS intakes have averaged about 80% of requirements (Figure 13). There are exceptions to this finding, including the Skylab missions of the early 1970s (28, 29), European flights to the Mir space station (30), and more recently some of the ISS missions (31). In the Skylab and Mir examples, crew participation in metabolic experiments has required consumption of balanced, controlled, eucaloric diets. As a result, crewmembers met their recommended energy intake requirements. It is difficult to determine whether the intakes on Skylab were related more to the requirement to consume the food or to the fact that the food was more palatable because of the additional variety available with frozen foods; however, increased palatability is obviously beneficial.

Figure 13. In-flight dietary intake of crewmembers in different space programs. Data are expressed as percentage of energy requirements predicted by the World Health Organization (17) and are mean ± SD. Apollo N = 33, Skylab N = 9, Shuttle N = 32, Mir N = 7, ISS E1-18 N = 26, E19-36 N =31. E = expedition. Apollo and Skylab data are from Bourland et al (32). Figure is adapted from earlier publications (2, 33), with additional published data included (6, 31, 34).

The International Space Station has accommodated 4- to 6-month missions dating back to 2000. During this time, many aspects of these missions have evolved and new exercise equipment, reformulations of
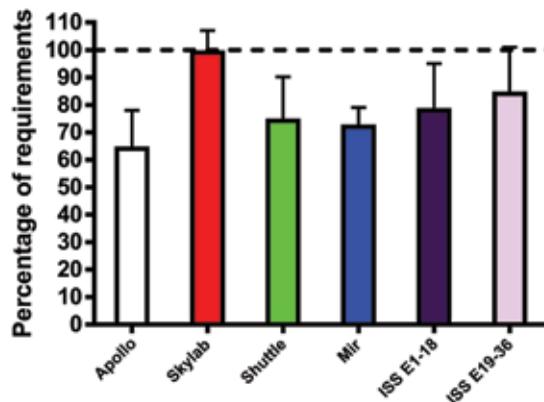
many space food items, and international foods from all partner agencies have debuted. These factors, coupled with the passage of lessons learned from one crew to the next, may have been responsible for our observation that many ISS crewmembers now consume recommended dietary intakes of energy, and also maintain body mass (6, 31).

In cases where energy intakes do not meet requirements, absent definitive causes, many potential explanations have been proposed (8, 23, 35). Anecdotal reports of appetite vary significantly, as indicated in a Russian study in which 40% of Mir crewmembers reported decreased appetite, 40% reported no change, and 20% reported increased appetite (36).

Food palatability is occasionally reported as a cause of reduced in-flight intake, and many anecdotal reports exist of changes in taste and aroma of food during flight (37-39). One hypothesis is that fluid shifts and congestion associated with the first days of microgravity can alter taste and odor perception. Other possibilities exist as well, including effects of atmospheric contaminants, stress, radiation, and psychological factors (37). Experimental research has not been able to clearly document changes in taste or olfaction during space flight or head-down-tilt bed rest (37, 40, 41), but it is hoped that ongoing ground-based and in-flight research on ISS will provide clarity.

When tongue taste perception was measured before, during, and after a 30-day –6° head-down bed rest period, subjects reported decreased appetite and lack of taste early in the bed rest phase (41, 42). By day 13 of the bed rest phase, for all tastes (sweet, salt, acidic, bitter), the threshold for taste sensitivity had increased. In contrast, a bed rest study in the 1990s found no changes in odor or taste perception after 14 days of head-down bed rest (43), suggesting that multiple factors are likely involved in this process. Additional studies of taste and smell changes during bed rest are ongoing, and will help expand the evidence base for this area.

Flight-related changes in gastrointestinal function may also occur. Fluid shifts, in combination with reduced fluid intake, would tend to decrease gastrointestinal motility. Gastrointestinal transit time has not been systematically studied during flight, but during 10 days of –6° head-down bed rest, mouth-to-cecum transit time was significantly longer than it was during ambulatory control periods (44). However, because the Skylab astronauts and others were able to maintain a eucaloric diet in space, hypotheses about inability to consume the requisite amount of food because of stomach fullness or other factors are not likely to fully explain decreased dietary intake during flight. Russian studies of gastrointestinal function during actual and simulated space flight, in humans and in animal models, have previously been reviewed (45). A common cause of reduced dietary intake during the first days of a mission (46) is space motion sickness (39, 46-49). The effects of space motion sickness typically pass after the first several days of flight, but the decreased dietary intake can extend well beyond the first week (35).

Implications for Inadequate Energy Intake

The obvious and immediate reason for concern about reduced dietary intake is the risk of body mass loss, and more specifically, loss of lean mass and bone tissue. Body mass losses of 1% to 5% of preflight body mass have been a typical finding in the history of space flight, although some crewmembers have been able to maintain body mass (6, 31, 50). In-flight and postflight losses of body mass are compiled in Figure 14 and Figure 15. Documented weight losses have occurred on short- and long-duration flights in both the US and Russian space programs (23, 51-53). Indeed, all crewmembers on Gemini, Apollo, Skylab, and Apollo-Soyuz Test Project missions lost body mass

(54); thus, ingestion of the prescribed energy intake on the US Skylab missions did not ensure maintenance of body mass (28). In one study of 13 male Shuttle crewmembers, body mass losses ranged from 0 to 3.9 kg (11). Body mass loss has been observed to reach 10% to 15% of preflight body mass (55). Crewmembers on ISS have shown similar patterns of mass loss during and after flight.
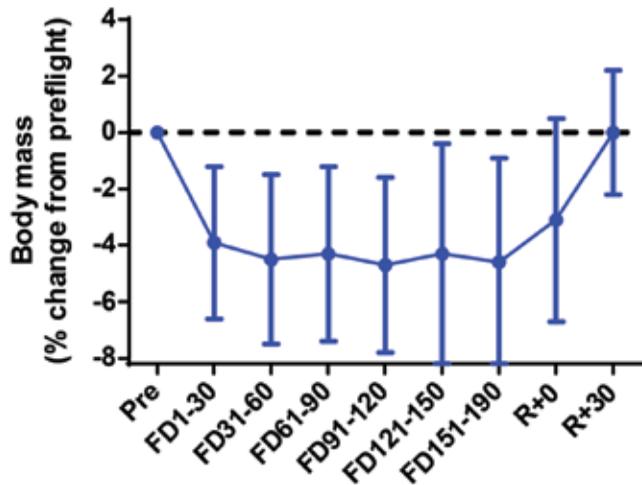
Figure 14. In-flight body mass measurement data from 55 ISS crewmembers. Pre = preflight, FDx = flight day x, R+x = x days after landing. Data are expressed as percent change from preflight values and are mean ± SD. Figure and data adapted from Zwart et al (7).
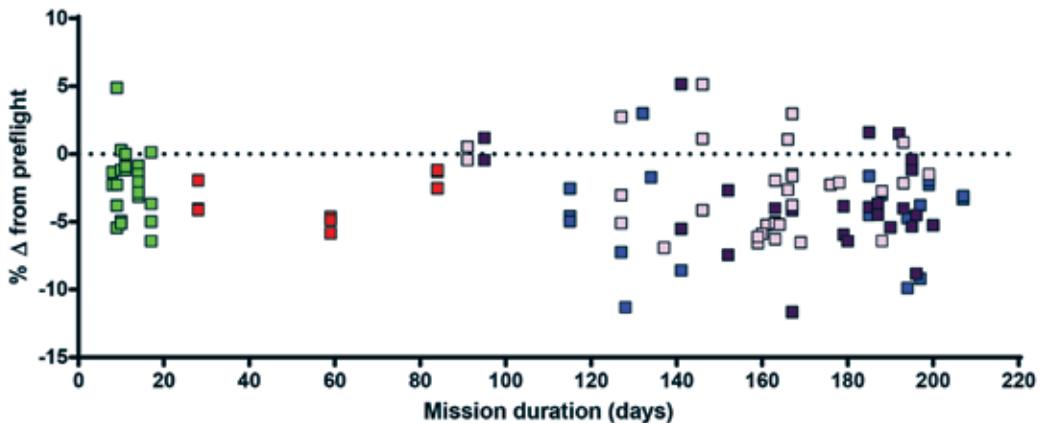
Figure 15. Changes in body weight on the day of landing relative to before flight. Data are expressed as percent change from preflight values. Each symbol represents 1 crewmember from a mission on the Space Shuttle (green, N=25), Skylab (red, N=9), Mir (blue, N=19), ISS Expeditions 1-18 (dark purple, N=26), or Expeditions 19-36 (light purple, N=31). Duration data have been adjusted slightly to ensure anonymity. Data updated from initial publications (2, 56).

Data from Apollo missions clearly document the relationship between energy intake and weight loss (Figure 16). Data relating reduced dietary intake during semi-starvation to loss of body mass were collected in 2 ground-based studies, not related to space flight. In the first study (57), subjects who consumed 580 kcal/d lost 7% of their body mass in 12 days and subjects who consumed 1010 kcal/d lost 11% of their body mass in 24 days. In the other study, starved subjects lost 9% of their body mass after 11 days, 15% by day 18, and 18% by day 43 (58).
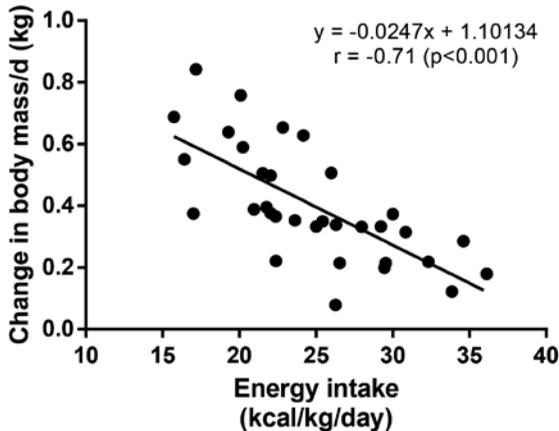
Figure 16. Relationship between energy intake (kcal/kg body mass/d) and weight loss (change in body mass/d, kg) during Apollo missions. N = 33. Data are courtesy of William Carpentier.

Only about 1% of the loss of body mass can be explained by loss of body water (19); most of the observed loss of body mass is accounted for by loss of muscle and fat tissue (10, 59). The water loss may be confounded by lean tissue loss, as metabolic water loss will be associated with depletion of glycogen stores and protein catabolism, both of which occur with inadequate intake. Inadequate energy intake is associated not only with loss of fat tissue, but also with decreased protein synthesis (60) (during space flight), increased protein catabolism (61) (during bed rest), and subsequent loss of lean tissue mass.

Besides the obvious concerns about body mass loss and dehydration (62), existing data suggest that many systems are affected by inadequate nutrient intake, including the muscle, bone, cardiovascular, and immune systems. The German Institute of Aerospace Medicine at the German Aerospace Center conducted a study jointly with the European Space Agency (ESA) to evaluate the impact of hypocaloric nutrition on multiple systems. A crossover design was used, with hypocaloric and eucaloric phases, and bed rest and ambulatory phases. Data from this study document the fact that undernutrition exacerbates the negative effects of bed rest on human physiology (61).

Undernutrition has also been found to impair cardiovascular performance (orthostatic tolerance) in controlled bed rest settings (63) and after space flight (William Carpentier, personal communication; see additional information in section 6, "Cardiovascular Health"). The mechanism for this energy-cardiovascular connection has been hypothesized to involve multiple functions of many endocrine factors, including insulin, leptin, and growth hormone (64).

Anecdotal reports from crewmembers on long-duration missions indicate that crewmembers who had lost a significant amount of body mass on orbit had an excess amount of rebound weight gain after landing, but in general, the data do not support this finding (2).

It is imperative that adequate resources be provided to support food consumption on exploration missions. A reliable food system must include a variety of palatable foods and the means (such as rehydration, heating, and cooling) to process them for consumption. Time (for meal preparation, consumption, and cleanup) is another limited resource that often hinders dietary intake during space flight. The use of freezers and refrigerators for food storage and preparation would provide a more palatable food system, which would increase dietary intake as well as provide added psychological support.

Deficiency of dietary energy intake leads to wasting and ultimately tissue breakdown, or even death. The loss of lean body mass during space flight is significant, and is

associated with increased proteolysis and catabolism related to metabolic stress (65). Inadequate energy intake can also have negative effects on bone, exacerbated by exercise (66, 67). This highlights the interaction between systems, and the fact that exercise regimens must be coordinated with energy provision.

It is difficult to predict the impact of suboptimal (or lack of) energy intake on otherwise healthy individuals. One issue is that the energy equivalent of the lost mass changes with time, as different body fuels are used at different times during semi-starvation (57, 68). With partial rations available (1000 cal/d), it is reasonable to expect that a person could survive for more than 4 to 6 months, potentially longer if the metabolic rate were to decrease because of decreased intake. If energy availability were restricted further, survivability would range between this amount of time and the 1 to 2 months possible with no food. These projections obviously include many assumptions, unknowns, and extrapolations. Data from 10 Irish Republican Army hunger strikers, who consumed water ad libitum but no energy, vitamins, or minerals, indicate that an average 25-year-old male could survive no longer than 60 days without energy (69, 70).

Other possible effects of long-term low intake of calories include decreased motor and cognitive function, each of which could impair an astronaut's ability to perform work-related tasks necessary for landing. According to military survival studies, astronauts would be expected to experience decreased endurance early on, and the decrease in strength would parallel the decrease in lean body mass (71). During total fasting, degradation of coordination, speed, and cognitive function would be evident within the first 2 weeks (71).

The metabolic condition of ketosis, which would be expected to result from starvation, not only would have metabolic effects (including decreased appetite), but might also affect other aspects of the mission (for example, the life-support systems might not be able to remove the ketones from the air). Ketoacidosis can obviously have negative effects on acid-base balance, which in turn can affect bone, muscle, and other systems.

It is speculated that a crew could survive on a spacecraft or planetary base for 40 to 60 days without food. With limited rations (1000 cal/d), a crew could survive 4 to 6 months (although physical performance capability might be severely degraded). The high-stress environment of a contingency during transit or on a planetary surface would likely exacerbate the basic effects of limited rations, and would shorten projections of survivability estimated from ground-based studies.

Insufficient dietary intake and subsequent loss of body mass are significant not only for crew health but also for medical operations and research studies, in which clear interpretation of essentially all other physiological data is impossible when subjects are malnourished. That is to say that virtually all space flight data collected on Shuttle, Mir, and many ISS missions are confounded by inadequate dietary intake. Investigators who have studied bone and muscle, cardiovascular function, immune response, and other systems during space flight cannot say to what degree undernutrition affected their findings.

While research may be warranted to better understand why astronauts typically do not consume 100% of their recommended intakes, recent data from ISS crewmembers clearly document that intakes can be met during space flight (31). In addition to maintaining energy intake and vitamin D status, in conjunction with exercise, these crewmembers maintained body mass; came home leaner, with less fat; and maintained bone mineral density at preflight levels (31). Additional details are provided in section 4, "Bone."

Studies of energy expenditure have been conducted only on short-duration (Shuttle) flights (11, 12). Whether the same trends continue on longer flights is not known (an ESA-sponsored study of energy expenditure on ISS missions is currently in development). The health implications of decreased energy expenditure need to be determined, and ways to prevent both in-flight loss and postflight gain of body mass need to be evaluated.

At least 2 approaches exist to controlling body mass and composition while studying human adaptation to bed rest: maintaining body mass (as is typically done in the US) or allowing subjects to lose total mass while keeping fat mass constant (and thus losing lean tissue). While this latter approach sounds intriguing, implementing it has proven very difficult, given the difficulties in measuring fat mass and adapting intake in a timely manner. Nonetheless, Biolo and colleagues have recently reported data suggesting that the more the fat mass increases during bed rest, the more lean tissue is lost, and that this loss is confounded by increases in oxidative and inflammatory damage markers (72). Altered fuel homeostasis has been documented in other bed rest studies (73) and animal studies (74, 75), but remains to be fully elucidated, in bed rest or space flight (75, 76).

Carbohydrate

Carbohydrates play an important role in the body because they supply the primary source of energy as well as a readily available source. This energy is oxidized and used by various organs and cells in the body, particularly the brain and red blood cells, which depend solely on carbohydrate for energy. The human body stores about 150 to 500 g of carbohydrate as glycogen, in the liver and skeletal muscle (77). Most of the body's glycogen is in skeletal muscle. Muscle glycogen stores are used mainly by muscle, whereas the smaller glycogen stores in the liver are used to maintain, store, and export blood glucose. Glycogen stores, especially those in the liver, fluctuate greatly during the day in response to food intake, and these fluctuations may be involved in the regulation of food intake (78). Liver stores of glycogen are depleted after 12 to 18 hours of fasting (77). In skeletal muscle, glycogen synthesis is triggered by a rise in insulin after the consumption of carbohydrates. De novo synthesis of glucose from non-carbohydrate precursors can and does occur in the body, if needed. This allows the liver to maintain adequate blood glucose concentrations. Insulin is required for the uptake of glucose into cells, and various transporter systems are found in different types of tissues that utilize glucose.

Requirements for carbohydrate in space are thought to be similar to those on Earth. However, to date, few investigations have been conducted on the effects of microgravity on the metabolism of dietary carbohydrate, and those studies have had conflicting results.

On the Shuttle, studies by German investigators showed no impact of 7 days of flight on glucose tolerance tests (79). A Russian study documented a reduction in fasting plasma glucose after 60 or 88 days of flight on a Salyut-Soyuz spacecraft complex, and a reduced peak of blood glucose in glucose tolerance tests (80, 81). Insulin resistance (lack of sensitivity to insulin) has been found to result from simulated weightlessness (bed rest) (73, 82-84). Using C-peptide excretion as a proxy, Stein et al found evidence of insulin resistance during actual and simulated space flight (85). Heer et al. documented that after 3 weeks of bed rest, glucose tolerance was altered for more than 4 days after reambulation (378). Efforts to maintain muscle mass (and presumably correct the insulin resistance) continue, but little research has been done to pursue this as a nutritional issue.

Suboptimal carbohydrate intake before and during space flight may have consequences for the crew's productivity and impede their ability to respond in emergency situations (86). Deficiency of carbohydrate leads to ketosis. A ketotic state would likely impair performance of crewmembers, as seen in studies conducted by the military (71), as well as increase renal stone risk secondary to reduced urinary pH (87-89). Other aspects of the mission would also be at risk (for example, the life-support systems may not be able to remove exhaled ketones from the air). Toxicity of carbohydrate has not been well studied, and would likely be an issue only because it would displace other nutrients (protein and fat) from the diet.

Few data are currently available to assess the impact of space flight on carbohydrate metabolism. Observations from space flight as well as ground-based bed rest studies show subtle changes in insulin secretion, insulin resistance, and glucose intolerance (83, 84, 90, 91). Even subtle changes in such important metabolic processes make it critically important to consider the likelihood, nature, and consequences of altered carbohydrate and insulin metabolism for exploration missions.

Fat (and Fatty Acids)

Fat is the most energy-dense of all the nutrients, and therefore is a major energy source for the body. Chemically, dietary fat is mainly in the form of triacylglycerols, which contain a glycerol backbone with as many as 3 fatty acids attached. Many types of fatty acids exist, including saturated, monounsaturated, polyunsaturated, and trans. Dietary fat assists in the absorption of fat-soluble vitamins and supplies the body with the 2 essential fatty acids, linoleic acid and linolenic acid. These essential fatty acids are necessary for growth and development as well as many other biochemical processes, including production of eicosanoids (physiologically active substances derived from arachidonic acid). Lipids, in the form of phospholipids, make up a large proportion of the structural components of the cellular membrane bilayer. Energy stored as fat is released in the process of fatty acid oxidation, and fat supplies more energy than any other macronutrient because of its higher content of carbon-to-hydrogen bonds. According to case studies, people following fat-free diets can exhibit symptoms of essential fatty acid deficiencies after only 1 month (92, 93).

While few, if any, studies have been conducted to look at dietary fat, plasma lipid levels, and related factors in space flight, voluminous data exist from routine medical examinations conducted before and after flight, along with annual medical exams, as reviewed previously (2). Contrary to the typical lipoprotein response to weight loss, low-density lipoprotein concentrations tended to increase in long-duration crewmembers who lost weight during the flight. This relationship seemed to return to normal by the subsequent medical exam (2).

Alterations in fuel homeostasis and regulatory hormones have been noted in space flight and ground-based studies. Bed rest studies have documented alterations in fuel homeostasis (94), including gender differences (73). Specifically, lipogenesis increased during bed rest, to a greater extent in women than in men. Additionally, men had increased carbohydrate oxidation (73). Other studies have shown inflammatory changes during bed rest, along with insulin resistance, leading to increased body fat and altered fatty acid metabolism (95). Given these data, and the insulin, leptin, and other endocrine changes noted in bed rest and space flight (15, 96, 97), changes in fuel homeostasis in bed rest clearly warrant additional investigation.

The role of omega-3 fatty acids in cancer prevention has been investigated in animal models of space flight radiation effects (98, 99). Not only do omega-3 fatty acids (in combination with pectin) show promise in alleviating cancer risk (98-102), but these fatty acids also have well-documented cardiovascular benefits. Abundant data show that eicosapentaenoic acid can successfully prevent muscle atrophy in other muscle-wasting circumstances, such as cancer or sepsis (103-112), indicating the likelihood is high that eicosapentaenoic acid will have the same beneficial effects on muscle atrophy during space flight or in ground-based analogs including bed rest.

Omega-3 fatty acids also have been shown to protect bone, in the general population (113-115) as well as in space flight analog studies, including bed rest and cell culture (116). Although omega-3 fatty acids have not been studied in a controlled fashion during actual space flight, a positive correlation was found between fish intake and bone loss in astronauts (116). That is, those who ate more fish lost less bone (Figure 17). These data provide additional evidence of the potential importance of fish oils as a countermeasure for muscle, bone, and radiation risks of space flight. Studies showing positive effects of omega-3 fatty acids typically look at intake of fish or other food sources of these nutrients (117-119). Studies of fish oil supplements added to typical diets often fail to document any benefit (120-122), thus highlighting the need for dietary modification, and not simply supplementation.
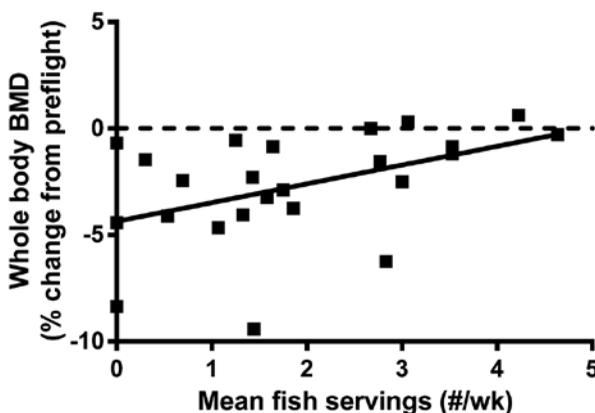
Figure 17. Relationship between fish intake during long-duration flight and whole-body bone mineral density loss after flight on ISS. Figure adapted from (116).

Cofactors in Energy Metabolism

Many micronutrients—vitamins and minerals—are involved in energy homeostasis, including thiamin, riboflavin, niacin, pantothenic acid, iodine, manganese, and even chromium. We previously reviewed sources, functions, deficiency symptoms, and concerns for space flight for these nutrients (2). In general, few data are available on these nutrients with respect to effects of space flight, or on availability and stability of these nutrients in the space food system. No specific concerns have been raised at this point, but ensuring adequacy of intake of these nutrients and understanding their metabolism, human requirements for them under space flight conditions, and their stability in the food system will be critical on exploration missions. Additionally, their nutritional status should continue to be monitored as we begin to embark on longer missions, including the initial 1-year mission on the International Space Station planned for 2015.

Astronaut Sunita L. Williams, Expedition 14 flight engineer, uses chopsticks to eat a meal near the galley in the Zvezda Service Module of the International Space Station.

3 Muscle and Protein

As the major structural component of all cells in the body, protein includes molecules that perform many essential physiological functions, serving as enzymes, hormones, transporters, and other important molecules. The total energy contribution of protein to the average diet is about 15%. The nitrogen in its amino-acid building blocks makes protein, along with nucleic acids, one of the major nitrogen-containing macromolecules.

Protein is one of the most important limiting factors when the body is deprived of energy, because essential amino acids are not stored in the body. A complete depletion of energy and protein reserves is said to be the cause of death from starvation. It is estimated that when 33% to 50% of total body protein is lost, death results (123). Loss of more than 40% to 50% of initial body mass is not compatible with life (71, 124). In one case report, individuals on a hunger strike lost 30% of their total body mass and 19% of total body protein before they died (69, 70).

Protein Intake

Maintaining a proper protein intake is vital, as both low-protein and high-protein diets can cause harm (and, at the extreme, death). A low-protein diet (below the recommended dietary allowance) for up to 4 weeks can decrease calcium absorption and cause increased secretion of parathyroid hormone in otherwise healthy subjects (125, 126). The impact of chronically low protein intake is not well understood; however, several studies suggest that low-protein diets are associated with loss of bone density (127, 128).

Actual intakes of protein during space flight typically exceed these recommendations (2), as shown in the table "Nutrient Intake Data" in the Appendices (page 134). European studies have shown that on long missions, reaching (or exceeding) nominal protein intakes is common, but that on short flights (Shuttle missions) protein intake is less than the recommended amount because of insufficient food intake (21). On ISS missions, on average, protein intake is more than adequate (Figure 18).
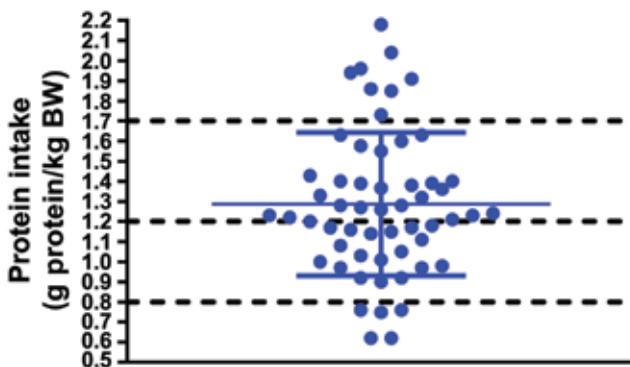
Figure 18. Protein intake during space flight on ISS missions (N=56). Each point represents an individual crewmember, and is their reported average intake over the course of the mission. Dashed lines represent space flight (and ground-based) protein intake requirements of 0.8 g protein/kg body mass, and the range of protein intakes (1.2.-1.7 g/kg) recommended by the American Dietetic Association, Dietitians of Canada, and American College of Sports Medicine for high-intensity athletes (130).

Some data suggest that during the recovery period after short-duration Shuttle flights, protein was a limiting nutrient, and that competition for substrate to replenish plasma proteins and muscle mass strains the system (129). This has not been tested experimentally, but it is clear that good nutrition is required for rapid return to optimal health.

Vitamin B_6

Vitamin B_6 comprises a group of 3 compounds and their 5-phosphates (P): pyridoxal (PL) and PLP, pyridoxine (PN) and PNP, and pyridoxamine (PM) and PMP (131). We previously reviewed vitamin B_6 basics (2). Weightlessness has been shown to reduce the cross-sectional area of muscle fibers and is associated with a change from type I to type II muscle fibers (132). As vitamin B_6 is stored mainly in muscle tissue (133), a decrease in muscle cross-sectional area could reduce the amount of the vitamin that is stored. Increased excretion of 4-pyridoxic acid (4-PA) during bed rest, a finding observed in short- (134) and long-duration bed rest studies (135), likely reflects this loss of muscle stores of vitamin B_6.

Given the changes observed in vitamin B_6 metabolism during bed rest, vitamin B_6 status during and after long-duration space flight warrants further attention. A deficiency in vitamin B_6 causes a decrease in the synthesis of serotonin and catecholamines, which has been shown to be associated with depression (136). Excess vitamin B_6 can lead to neuropathy (137-139).

There is no evidence that vitamin B_6 status changes during long-duration space flight; however, we have shown that crewmembers who have experienced vision-related issues have higher blood concentrations of cystathionine and 2-methylcitric acid than crewmembers who did not experience such issues (140). Although increased levels of these metabolites do not point to a vitamin B_6 deficiency, they do suggest there may be a perturbation in the 1-carbon metabolism pathway, which is dependent on folate, vitamin B_{12}, and vitamin B_6.

Muscle Loss and Protein Turnover in Microgravity

Exposure to microgravity reduces muscle mass, volume, and performance, especially in the legs, on both short (28, 141-146) and long flights (28, 141, 147-151).

As with most physiological systems, a variety of techniques are used to quantify changes in different aspects of muscle. These include functional muscle performance/ exercise tests, muscle biopsies to evaluate cellular changes, magnetic resonance imaging (MRI) of muscle tissue, and tracer kinetic studies to evaluate changes in protein metabolism. Each technique provides a different perspective and unique information. The picture becomes more complex when results from models and analogs (including nonhuman ones) are compared with those from space flight. As a result, the topic of space flight effects on muscle is very complex, and defining and understanding the big picture is difficult, to say the least. This topic has been reviewed (142, 152-156), and the difficulties of interpreting findings across the literature have also been noted (146, 152, 155).

Basic clinical testing includes determinations of circulating proteins. Blood concentrations of total protein and albumin were elevated at landing after Skylab missions. Urinary albumin has been shown to be reduced during space flight and bed rest (157-159). Measurements of urinary albumin excretion, which is typically

low in healthy individuals, have not been reported after landing. Potassium and nitrogen balances became increasingly negative throughout the Skylab flights, but urinary creatinine (a measure of muscle mass) did not change (29, 160) despite losses of leg volume (28, 161). Nitrogen balance has also been shown to be negative during Shuttle flights (162).

Evaluation of plasma and urinary amino acid levels often does not provide a clear picture of muscle or even protein metabolism, but in some cases, these are among the limited available data. An increase in plasma amino acids was noted in cosmonauts after short (2-d) and long-duration (63-d) flights (163, 164). Limited Shuttle flight data indicate a tendency for plasma branched-chain amino acids to be increased during flight, relative to preflight levels (165). Data from short-duration Shuttle flights have revealed little or no change in urinary amino acid profiles (26), but data from the Apollo and Skylab missions showed increases in urinary excretion of the amino acid metabolites creatinine, sarcosine, and 3-methylhistidine (166), suggesting that contractile proteins of skeletal muscle are degraded in weightlessness.

The amount of protein in the body or individual tissues is affected by the balance of protein synthesis and protein catabolism. Studies to understand changes in body protein, ideally, will include measurement of both of these factors in addition to turnover. Measuring these factors is not easy, and the results are variable (151, 167), but having the complete picture is critical. For example, although both decreased protein synthesis and increased protein catabolism will yield a net loss of muscle, the mechanisms and countermeasures for the 2 processes are quite different.

Turnover studies with stable isotopes indicate that during short-term space flight, whole-body protein turnover and protein synthesis increase, and a greater percentage increase occurs in protein breakdown (162, 168). The increase in synthesis is hypothesized by Stein et al (156, 169) to be related to physiological stress, as indicated by generally (but not consistently) increased urinary cortisol during flight (15, 19, 97). These findings are similar to those in catabolic patients undergoing metabolic breakdown. Decreased prostaglandin secretion has also been implicated in the loss of muscle tissue during space flight, secondary to decreased mechanical stress on muscle (97). On long-duration Mir flights, conversely, investigators noted decreased rates of protein synthesis (12), secondary to reduced dietary energy intake (60).

Ground Analog Studies

In humans, bed rest is the most common model used for studying changes in muscle and protein during disuse. Some studies have also used unilateral limb suspension (ULLS) as a model for these changes, allowing subjects more freedom, while significantly reducing the costs of such studies. Other human models have also been tested, including "dry immersion," as reviewed by Navasiolava et al (170). All of these models provide a means to collect data that would be difficult if not impossible to collect during actual space flight, on a larger group of subjects. However, it is important to remember that the model system may not, and likely does not, provide an exact replica of the physiological changes during space flight. Untreated bed rest and ULLS both result in loss of muscle mass and strength, as nicely reviewed by Narici and de Boer (152).

As mentioned earlier, muscle loss in space may be related to changes in turnover of protein in the whole body. Many studies document a decrease in protein synthesis during bed rest (171-178). Evidence of increased protein catabolism during bed rest is limited, as reviewed by Bodine (155). There are 2 key confounding, and intertwined, issues (among others) with this area of research: energy intake and inflammation.

Energy consumption during space flight is generally not controlled; that is, astronauts are allowed to consume as much, or as little, as they desire. Historically, this lack of control has resulted in underconsumption, as described in section 2, "Energy and Fuel Metabolism." The prospect of comparing the controllable ground-analog model with actual space flight, in which underconsumption can occur, presents a study design issue. Investigators typically either seek to maintain energy intake and body mass of the subjects during bed rest, or restrict calories to recreate the hypocaloric intakes of space flight. Other studies allow subjects to regulate energy intake voluntarily.

It should be noted that, because energy expenditure decreases (about 15%) during bed rest secondary to decreased activity (14, 179), essentially all studies reduce energy intake during bed rest to some degree. Furthermore, because protein is an energy-containing nutrient, it is impossible to alter caloric intake without altering either protein intake or the relative contribution of protein to overall energy intake. This becomes critical in nutritional countermeasure studies, in which provision of additional protein and/or amino acids is often a focus.

Restricting energy consumption to match earlier findings from space flight concedes the point that astronauts may be able to maintain dietary energy intake during flight. This was observed during Skylab and EuroMir missions, albeit in a controlled fashion (ie, these crewmembers were participating in metabolic studies and were required to consume the planned menu) (180, 181). More recently on ISS, crewmembers have been able to maintain energy intake at >90% of predicted requirements. Coupling this energy intake with heavy resistance and other forms of exercise, crewmembers maintained body mass, returned with an increased percentage of lean body mass, and maintained bone mineral density (31). This study is described in more detail later in this book, but it is mentioned here because it documents that crewmembers can consume adequate energy intake without their diet being strictly controlled.

Inadequate caloric intake results in 2 significant effects on protein and muscle. Initially, breakdown of glycogen in muscle will reduce muscle volume, in large part because of the water content associated with stored glycogen. Second, the body will catabolize protein for energy. Thus, studies of hypocaloric subjects will inherently be confounded by this situation. As detailed below, provision of supplemental protein or amino acids is an oft-tested countermeasure for muscle loss, but it is plausible that provision of energy in any form will help maintain lean (and other) tissues in this metabolic environment.

Muscle loss has been observed in bed rest and other analog studies, even when energy intake and body mass are maintained. It has been argued that in individuals who lose muscle under those conditions, maintenance of body mass during bed rest leads to a chronic, low-level inflammatory response, which can subsequently lead to increased fat mass along with muscle loss (72, 182, 183). This argument has led to attempts to reduce caloric intake to the point where fat mass is not changing (although body mass decreases, related to loss of muscle tissue). This is a difficult prospect at best, given that body composition is measured (typically by dual-energy x-ray absorptiometry) infrequently (eg, every 2 wk), and thus any corrections lag behind, or may overshoot, before the next determination.

The ideal answer for how to control body (or fat) mass during bed rest remains under debate. Nonetheless, as with any scientific study, it is critical to know the limitations and implications of every study design and, if possible, to relate studies with these limitations to other studies in the literature. One necessity for making the findings of such studies useful to readers of scientific papers is body mass data, which we suggest should be reported in every published bed rest study.

Beyond the controllable factors, other issues exist that can affect bed rest study results. Variability in stress levels can be a key factor for many physiological systems, but particularly muscle. An increase in stress level, as indicated by increased concentrations of cortisol in blood plasma and urine, is typically associated with the initial days of space flight. In many cases, urinary cortisol excretion returns to preflight levels after 5 to 9 days, although this phenomenon has yet to be fully characterized or generalized to all crews. Ground-based studies have the potential for stress to increase, but this is not an entirely consistent finding. Some studies have shown no change, or even a downward trend, in cortisol excretion during bed rest (184). As seen with studies of energy metabolism, administration of exogenous cortisol or thyroid hormone induces metabolic stress, which may produce a more accurate ground-based model of protein metabolism during space flight (16, 185-187). Ground-based rodent studies generally show increased proteolysis along with reduced protein synthesis (151), a pattern similar to that seen in studies of humans during flight, described above.

Muscle Loss Countermeasures

Mechanical

Exercise is the most common first-pass approach to maintaining muscle mass and strength (142, 154, 188-190). The exercise regimens tested as countermeasures to date have generally not succeeded in maintaining muscle mass or strength (or bone mass) during space flight (190). On Mir flights, crewmembers differed significantly with respect to in-flight exercise frequency and intensity (related to such factors as mission requirements and personal habits). However, losses of leg muscle volume, detected immediately after flight by magnetic resonance imaging, were almost 20% in all subjects (191). Similar findings (wide variations in exercise, lack of difference in bone loss) have also been documented for bone loss (55).

Many types of exercise protocols have been proposed to aid in the maintenance of both muscle and bone during flight (175, 192-203), but these have yet to be fully tested on orbit. Given that ISS crews use multiple exercise devices, studies ideally will look at exercise protocols that use more than 1 device. Combined resistance and aerobic exercise protocols have shown promise for protecting muscle (and the cardiovascular system) in bed rest (204, 205); space flight testing is underway.

Whole-body vibration alone or superimposed on resistive exercise is a rather new concept and has received much attention recently in the hope that it can provide a viable musculoskeletal countermeasure, particularly when strenuous exercise might increase the risk for injury, for instance in older people (206-213). However, the efficacy, vibration dose, frequency, and duration of whole-body vibration exercise need further research. As mentioned earlier, mechanical stimulation is a prerequisite to avoid muscle and bone degradation. According to the mechanostat theory by Frost (214, 215), to keep up muscle and bone mass and strength, a certain individual level of mechanical stimulation has to be achieved. Lowering or increasing that level leads to respective degradation or increase. For whole-body vibration training, the vibration magnitude seems to be one of the key factors. Vibration magnitude is vibration frequency (Hz) times the amplitude or displacement (mm) (211). This is most likely the reason why mere whole-body vibration training to the musculoskeletal system with a frequency of 20 Hz has not always been demonstrated to be effective for muscle and bone (216). Superimposing whole-body vibration training on resistive exercise seemed to be more effective. This was done in 2 bed rest studies with durations of 56 and 60 days and

young, healthy male subjects (210, 213). When whole-body vibration training was added to resistive exercise, it was able to attenuate muscle and bone atrophy and lumbar spine deconditioning, and to prevent fat accumulation in vertebral marrow (210, 217-220).

Pharmacological

Testosterone has also been suggested as a muscle (and/or bone) loss countermeasure, on the basis of data from animal models (221, 222) and data documenting a reduction in testosterone concentrations during flight in humans, animals, and cellular models of space flight (223-230). A potential confounding factor is the drop in testosterone that has been observed in exercising bed rest subjects (but not controls) (231).

The first in-flight testosterone data from human space flight were from 4 astronauts on Space Shuttle mission STS-55, which flew in 1993, and from 3 astronauts on Skylab 4, an 84-day mission (232). On the Shuttle mission, after 4 or 5 days of flight, circulating testosterone levels were decreased relative to preflight levels, when measured in serum, saliva, and urine. Serum cortisol, cortisol biorhythms, and dehydroepiandrosterone-sulfate concentrations in these 4 astronauts were unchanged during flight (228, 233). A significant confounding issue is that these crewmembers were consuming only about 60-85% of their basal metabolic energy requirements during the flight (21). Estimates of space flight energy requirements typically use an activity factor of 1.7 (that is, 1.7 x basal metabolic rate) (2). This factor is based on data documenting that total energy requirements are unchanged during flight (11), or in some cases are even increased with heavy exercise (12), relative to before flight. Even if lower estimates of activity were used, the result would reveal significant energy deficit in crewmembers on the STS-55 mission, especially during the days of sample collections (21). Indeed, energy intake, which was very carefully documented on these missions, was below even basal requirements. Energy deficits, both short-term and long-term, are associated with lower circulating testosterone (free and total) (234-236). Thus, the discrepancy between the long-duration data presented here (Figure 19) and the earlier reports of effects observed during the first week of flight could be explained simply by inadequate energy intake.

Recent data show that testosterone and related hormones are unchanged by real or simulated weightlessness, apart from transient effects on landing day (237).

The Skylab data include urinary testosterone from 3 crewmembers; at 2 in-flight data points, excretion was increased relative to the preflight period (232, 237). Plasma data from the 3 Skylab missions (N=9) are reported to have shown
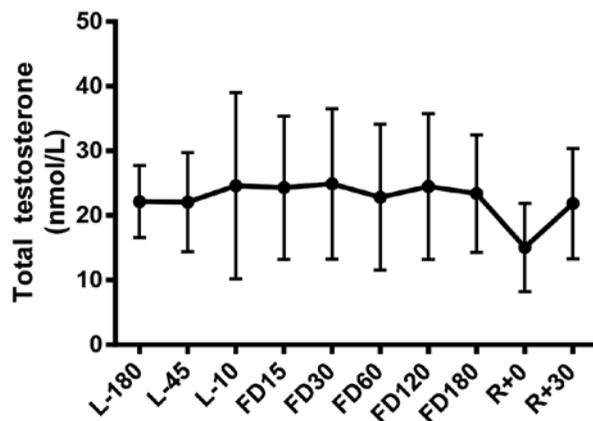

Figure 19. Total serum testosterone concentrations (mean ± SD) before (L-x), during (FDx), and after flight on ISS. Although circulating concentrations decreased significantly after flight (at R+0), no other time point differed significantly from the preflight mean. N=15. Data are from Smith et al (237).

"a trend toward lower values after the mission" (232). Although we do not have urinary testosterone data on all crewmembers, these reports from the 1970s confirm the findings from ISS (237).

Bed rest analog data from a number of studies demonstrate that bed rest has no effect on circulating testosterone concentrations (174, 231, 237-239). Consistent decreases in serum testosterone were observed after subjects had been in the bed rest facility for 7 days (while they were still ambulatory) and then another decrease occurred when testosterone was measured 5 days after re-ambulation. The pre-bed rest change is likely related to stress and decreased ambulation while subjects were in the bed rest facility, and the post-bed rest change was probably related to body fluid shifts during and after bed rest. No changes in testosterone occurred during bed rest (237).

Bed rest studies have generally shown no effect of bed rest on circulating total or free testosterone (174, 231, 237-239) in sedentary subjects. Bed rest subjects, in most models, are required to consume energy at a level to maintain body mass. If energy deficits are indeed part of the observed decrease in testosterone during the Shuttle flights previously reported, this may also explain the difference between those flight data and bed rest study data, reported herein and elsewhere. Although we showed an intermittent decrease in total and free testosterone in bed rest subjects with or without an artificial gravity (ie, centrifugation) protocol (134), this study had combined the 2 pre-bed rest collection sessions. When these sessions were analyzed separately because of our results in the later bed rest study, it turned out that testosterone concentrations were indeed higher only at the first data collection point (BR-10) than during or after bed rest (237) (Figure 20).

Figure 20. Total serum testosterone concentrations (mean ± SD) in subjects before, during, and after bed rest with (dashed line and open circles) or without (solid line and filled squares) an artificial gravity countermeasure. These data have been published (134), although the pre-bed rest data (BR-9, BR-1) were combined as an average in the original manuscript. In this figure, the 2 pre-bed rest data points are graphed separately, as reported in (237).

One criticism of sedentary bed rest studies as an analog for space flight is that astronauts are not sedentary, especially on long-duration missions, when they exercise extensively. Wade et al reported that in a 4-week study, bed rest subjects with intensive exercise protocols had lower non-fasting circulating plasma testosterone concentrations than non-exercising bed rested controls (231). They reported a small loss of (non-fasting) post-breakfast body mass (231, 240), and reported that caloric and liquid intakes were designed to maintain body mass. Despite the exercise, which was described as including an expenditure of 214 or 446 kcal/d (5 times a week), actual intakes in the exercise groups were only 155 or 212 kcal/d greater than those of the no-exercise group (240).
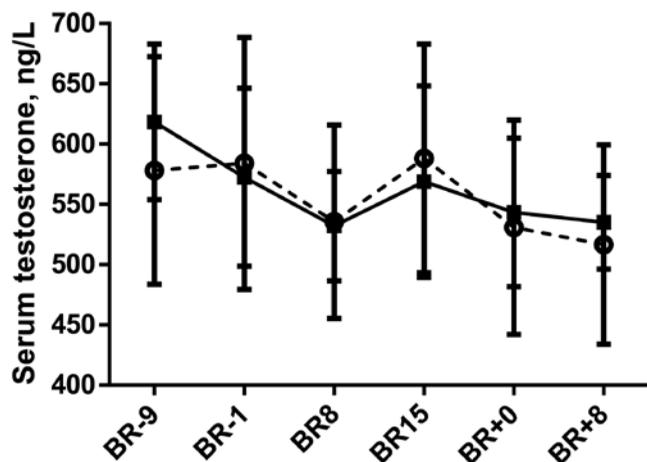
In an earlier 30-day study, Zorbas et al showed that serum testosterone decreased during bed rest only in trained subjects, whereas it did not change in untrained subjects during bed rest (241). Interestingly, when conditioned subjects were "hyperhydrated" by saline ingestion during bed rest, testosterone did not change relative to the pre-bed rest period. In a shorter, 3-day bed rest, no differences in plasma testosterone were observed before or after exercise, in typically untrained or trained individuals, either cyclists or weight trainers (242). The astronauts in the study reported herein were relatively fit before flight and exercised heavily during flight, using treadmill, cycle, and resistive exercise devices. Clearly, the interrelationship of energy balance, exercise, stress response, and endocrine function warrants further evaluation to better understand the adaptive responses to space flight.

As reviewed by Tou (243), in studies of rats with sample collections after space flight, serum (244) and urinary (245) testosterone were generally decreased relative to the preflight period (246). Unfortunately, in-flight biological samples are typically not available, given the difficulties with collection procedures in the microgravity environment. These postflight conclusions are consistent with data reported on landing day after a short-duration space flight (237). In ground-based rodent models, short-duration (7-12 d) unloading generally results in reduced circulating testosterone concentrations and an associated loss of bone and muscle mass (221, 222, 247). Unloading of longer duration (6 wk) in rats resulted in impaired spermatogenesis, but had no effect on circulating testosterone concentrations (248). Similarly, the production of testosterone by rat testes after actual space flight is diminished, as is response to stimulation by luteinizing hormone (249). Contradicting these findings, another study showed no change in circulating testosterone in suspended rats after suspension for 1 or 3 weeks, but an increase in testosterone of suspended animals after 8 weeks, despite reduced testicular weight (250). One critical confounding factor in the hindlimb-suspended rat model is that not all studies take (surgical) precautions to prevent ascension of the testicles into the abdominal cavity, which can significantly affect testosterone production, and interpretation of the study. Some, but not all, studies have accounted for this, and this limitation contributes to inconsistencies in the literature.

Rotating cell culture vessels have also been used as an analog of weightlessness, with some limitations, as with all analogs. Cultured testicular fragments exposed to this environment, compared with static 1g cultures, have maintained cellular architecture and have increased both proliferation and testosterone secretion (251), but with altered testicular physiology (252), including impaired Leydig cell responsiveness to luteinizing hormone stimulation. Whether the lack of change in circulating testosterone observed in the studies reported herein obscured alterations in testicular physiology is unclear, but it seems imprudent to make that leap without additional data.

Exogenous testosterone administration in humans during bed rest studies has maintained muscle mass and protein balance, but with no effect on muscle strength (239). Administration of testosterone to suspended rats mitigates muscle and bone losses (221). The bone data in rats are confounded by differential effects on growing and adult rats (253), but these results are of interest nonetheless. Testosterone administration to elderly individuals has shown that the bone response to testosterone depended on the initial circulating testosterone concentrations (254). That is, subjects who had normal blood concentrations of testosterone had less or no response to testosterone administration. Given these data, there is little rationale for providing testosterone during flight to mitigate bone loss.

Hypergravity, induced by centrifugation, has been shown to result in increased urinary testosterone excretion in monkeys (225), as well as in rats (245, 247). Hypergravity has also been found to affect tissues of rats and some other endocrine variables, but increased gravitational force had no effect on circulating testosterone (244). On the basis of these data, authors have suggested that the response to gravity is roughly linear, from hypergravity (increased), to unit gravity, to microgravity (decreased) (225, 244). Intriguing as this concept may be, the data presented herein do not support it.

As is understandable, the proposed use of exogenous steroids is somewhat controversial. Muscle physiologists argue that despite the lack of change in endogenous steroids, exogenous androgens may prove a viable countermeasure nonetheless. Treatments with such androgens have been reported to improve physical performance, muscle mass, and muscle strength in both young athletes and older sedentary men (255). The interaction of endocrine factors, aging (including middle age), the space flight environment, and the use of exercise to replace loading is not well understood.

In summary, circulating testosterone and related hormones are unchanged by real or simulated weightlessness, apart from transient effects after flight. Nonetheless, as we contemplate space exploration beyond low Earth orbit, endocrine data will be critical for understanding human adaptation in this unique environment, and potentially for helping to counteract the negative effects of space flight on the human body.

Nutritional

Use of protein and amino acid supplementation has long been studied as a potential means to mitigate muscle loss associated with space flight (82, 187, 204, 256-260), but results have been inconclusive at best. Oral doses of branched-chain amino acids had little effect on leg-muscle protein kinetics in ambulatory male subjects (261), whereas feeding a bed rest group adequate energy with excess protein reversed nitrogen losses (176). However, feeding Skylab crewmen energy and protein equivalent to those given to the bed rest group did not prevent negative nitrogen balance and loss of leg muscle strength during flight (148, 160, 161). In another bed rest study, a leucine-enriched, high-protein diet failed to mitigate muscle loss, and in some sites exacerbated loss (258). It remains unclear whether nutritional measures beyond the consumption of adequate energy and protein would be beneficial in reducing muscle atrophy.

In a 2011 review, Stein and Blanc evaluated the literature from bed rest studies (262), and found that the effect (or lack thereof) of amino acids on muscle depended greatly on protein intake and energy provision. Specifically, if nominal protein intake (ie, in both treatment and control groups) was at levels greater than 1.1 to 1.2 g protein/kg body mass/d, then supplemental amino acids had no effect. If control subjects were provided with ≤0.8 g protein/kg body mass/d while the supplemented group consumed >1.0 g protein/kg body mass/d, then the supplement appeared to have an effect. If further research is conducted in this area, the issue of study design, with particular reference to protein and energy intake of all groups, needs to be carefully assessed. The bottom line seems to be that if crewmembers consume enough protein and energy, then supplemental amino acids (or other variants of protein supplementation) provide no benefit (and as reviewed in the next subsection, "Protein and Bone," they may actually be detrimental). Thus, the primary countermeasure against muscle loss remains adequate energy intake, which will no doubt include protein, but protein supplements are not required. In fact, protein can have negative implications for other systems, bone in particular.

Debate continues between nutritionists and exercise physiologists on the importance and benefit of protein intake, amount and type of protein intake, and even timing of protein intake on muscle. It is our contention that these studies are often too short (if not simply a single bout of exercise) to allow an understanding of long-term effects and adaptation, and are often not completely controlled with respect to treatment groups. Protein (or amino acids) provides not only a nitrogen source, but moreover, an energy source. Studies often compare protein consumption with fasting, without evaluating whether a balanced meal would offer similar benefit. The summary above, regarding the view of amino acid supplementation during bed rest, certainly gives reason for concern about studies of protein and exercise, with or without bed rest. Thus, fully balanced, long-term studies are required in order to conclusively define the effect of protein intake and its timing on muscle. Unfortunately, these studies require multiple treatment groups, which drives study costs to levels that are typically not available. We will not review this field of literature here, but we refer interested readers to the Muscle Space Flight Evidence Book.

Protein and Bone

The interrelationship of protein and bone health is complex, and often seemingly contradictory. In certain populations (such as growing children), protein is essential for bone growth. However, in some cases, high protein intakes can be detrimental to bone (263), a fact confounded by the type of protein (and amino acids) consumed and by their relation to other dietary factors (264, 265).

High-protein diets lead to hypercalciuria, and increase the risk of bone fracture and the risk of renal stone formation (265, 266). In one 5-year study of 120 men, the relative risk of stone formation on a restricted protein (52 g/d) and salt (50 mEq/d) diet was found to be half that of men on a calcium-restricted diet (400 mg/d) (267). The reason for the decreased risk of renal stones on a low-protein diet is not well understood, but several potential mechanisms have been postulated. It is generally well accepted that high-protein diets induce hypercalciuria, and this can contribute to formation of calcium oxalate or calcium phosphate stones. One hypothesis to explain protein-induced hypercalciuria is related to the "acid-ash" hypothesis that excessive intake of animal protein provides excess sulfur-containing amino acids that are metabolized to sulfuric acid (268, 269). Since bone is a large reservoir of base, it can be broken down to provide carbonate or phosphate to neutralize fixed acid loads. Furthermore, low urinary pH decreases urinary excretion of citrate, which is a potent inhibitor of stone formation. In addition, dietary animal protein represents a rich source of purines that may raise uric acid excretion, which could increase the risk of forming uric acid stones (270).

Protein-induced hypercalciuria may also be detrimental to bone (271). Some studies show that high-protein diets increase intestinal absorption of calcium (272), but this has not been widely accepted. The key to understanding the interrelationship of protein and bone may lie in understanding the complexities of these types of studies, which may require a full accounting of many nutrients and environmental factors (273, 274).

Several studies show that animal protein increases acid load more than does vegetable protein because of the higher sulfur content per serving of food. Vegetable protein itself does not necessarily have less sulfur per gram of protein, but a larger mass of foods containing vegetable protein would have to be consumed to get the same amount of protein as from foods containing animal protein. It can be assumed that foods containing vegetable protein contain less sulfur than foods containing animal protein. In studies

with controlled dietary intakes with varying sulfur content, diets consisting of animal protein yielded greater urinary calcium excretion and lower urinary pH than similar diets consisting mainly of vegetable protein (275). The results of another study comparing the effects of 2 sources of protein (meat and soy protein), with and without additional supplementation with sulfur amino acids, indicated that dietary meat elicited a greater positive association between protein intake and urinary calcium, sulfur, ammonia, and titratable acids than dietary soy elicited (276). When the soy diet was supplemented with sulfur amino acids, urinary calcium and acid excretion increased. Conversely, the addition of dietary potassium (as fruit or K^+ supplement) to both diets decreased urinary calcium and acid excretion (276). Other studies have shown that greater amounts of protein or higher ratios of animal protein to potassium are more detrimental when bone health is already compromised (such as during bed rest, and potentially during space flight) (277, 278).

Dietary intake of protein, specific types of protein, and patterns of acid and base precursors have recently been associated with the concentrations of urinary markers of bone resorption during bed rest (277, 279, 280). In one study with male identical twins, the relationships between acid and base precursors in the diet and markers of bone and calcium metabolism during bed rest were investigated (281). With respect to dietary intake patterns, a strong positive correlation existed between markers of bone resorption (n-telopeptide [NTX], deoxypyridinoline, and pyridinoline) and the ratio of animal protein to potassium intake during bed rest. A positive correlation existed between urinary NTX excretion and the ratio of animal protein to potassium intake during 4 weeks of bed rest (277). No relationship was found between the ratio of vegetable protein to potassium and markers of bone metabolism. There tended to be a positive association between these variables before bed rest and during weeks 1 and 2 of bed rest, but the relationship was not significant, likely because of high variability among the population and small sample size (277). These results document that the ratio of animal protein to potassium intake was less related to bone metabolism markers in the group of subjects who exercised and more related to bone markers at the end of bed rest, when calcium excretion was highest. The results support the argument that calcium status could have an important role in determining the effect of protein on bone. If calcium is being resorbed from bone, then acid load can have a more detrimental effect on bone, similar to what has been observed in other studies of the effect of high-protein diets on bone (263, 265).

The idea that the levels of acid and base precursors in the diet can affect bone and calcium metabolism is supported by the results of studies testing the ability of a supplement containing essential amino acids and carbohydrate (45 g/d essential amino acids and 90 g/d sucrose) to mitigate muscle loss (278). The supplement contained 1.5 g methionine, which is about 1.13 times the recommended daily intake (supplementing the amount of methionine provided in the diet). The sulfur in methionine is converted in the body to sulfuric acid, and thus methionine is an acid precursor in the diet. It was evident that more methionine was broken down than was used by the body because urine pH decreased in the amino acid-supplemented group (Figure 21). It was hypothesized that this low-grade metabolic acidosis (282) contributed to the higher urinary concentrations of bone resorption markers and calcium excretion in the supplemented group.

In a separate study, 13 volunteers were subjected to 60 to 90 days of 6° head-down-tilt bed rest (283). Net acid excretion, as determined by dietary acid and base components, was positively correlated with NTX during but not before bed rest (Zwart et al, unpublished data). Net acid excretion has also been associated with calcium loss using meta-analysis techniques (284).
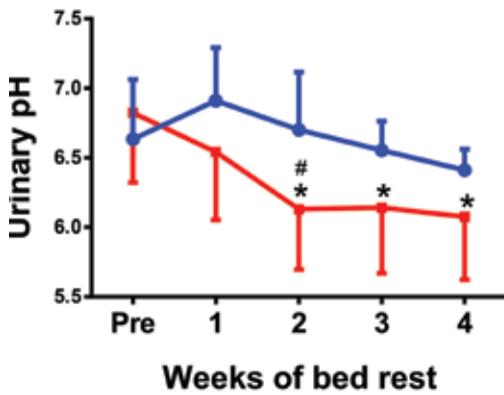
Figure 21. Urine pH (mean ± SD) of amino acid-supplemented (red) and placebo (blue) groups during 4 weeks of bed rest. *Significantly different from before bed rest (Pre), $P < 0.05$. #Significant difference between groups, $P < 0.05$. Figure is from Zwart et al, J Appl Physiol 2005 (278).

The risks associated with protein intake come from deficiency or excess. Deficiency of protein leads to muscle loss, weakness, wasting, tissue breakdown, inability to perform the job (including getting out of the spacecraft), and ultimately death. Low-protein diets can have negative consequences for bone (125, 285-287). Excess protein exacerbates increased excretion of calcium and the risk of renal stone formation, and is detrimental to bone. Specific amino acids may additionally increase these risks.
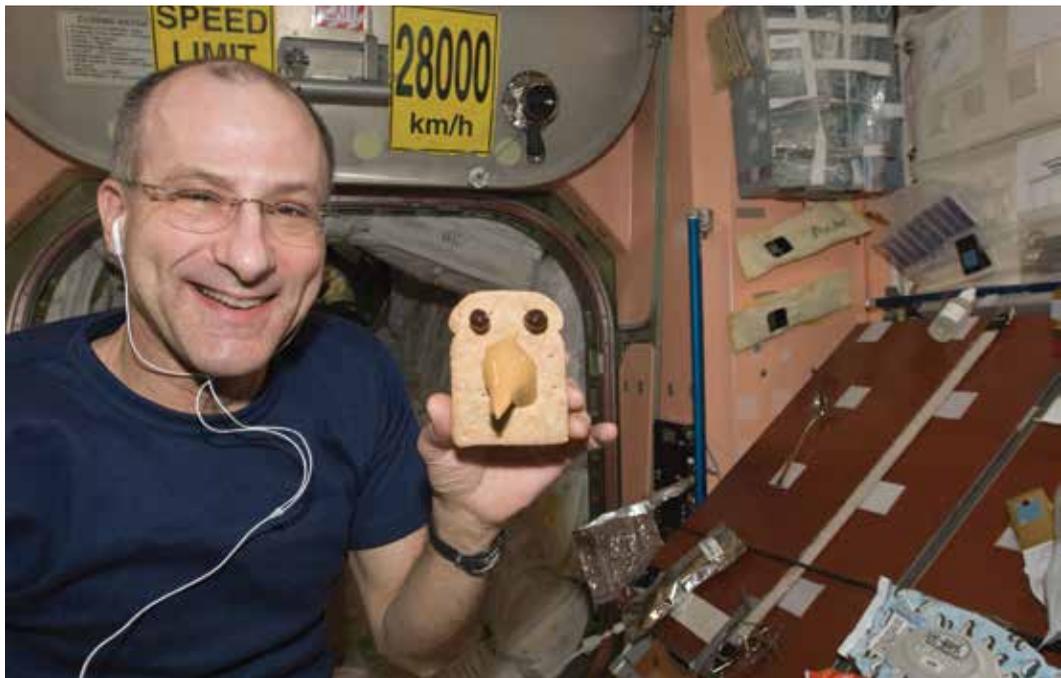
View of Expedition 30 Flight Engineer Don Pettit in the ISS Node 1 with an edible "friend" composed of flat bread, peanut butter and chocolate-covered espresso beans.

4 Bone

Whereas nutrition is critical for virtually all systems, the interaction of nutrition with bone is perhaps more extensive and complex than most of its interactions with body systems. Bone is the body's reservoir of calcium, which provides structure and strength to bone, but also provides a ready resource to maintain blood calcium levels during periods with insufficient dietary provision of calcium. Several nutrients are required for the synthesis of bone, including protein and vitamins D, K, and C.

Multiple risks are associated with bone loss during space flight. Almost immediately upon entering weightlessness, bone resorption increases, and calcium (and other minerals) are released into the blood and urine. This increases kidney stone risk on short missions, and on longer missions, chronic bone and calcium loss can increase risks to bone health both in the near term (eg, risk of fractures) and in the long term (eg, risk of osteoporosis-like bone degradation). The International Space Station provides the opportunity for relatively large numbers of crewmembers to fly on missions of 4 to 6 months, with even longer missions being planned. At current capacity, 6 crewmembers are on board at a time: 3 Russian cosmonauts and 3 astronauts from the other partner agencies (Canadian, European, Japanese, and US space agencies). This number of crewmembers has allowed the effects of space flight on bone physiology to be documented, along with the testing of countermeasures aimed at counteracting bone loss.

We provide here an overview of bone and calcium changes that occur during space flight, and we review the research efforts made to understand bone loss, including more detail about specific nutrients. We provide an update on recent countermeasure studies, which have started to show progress toward meeting the challenge of mitigating risks associated with bone and calcium changes during flight.

Bone Loss

Bone is lost during space flight, primarily from weight-bearing bones. This was first documented on Skylab missions (288, 289), and later on Mir (290-293) and ISS missions (31, 50, 294). Attempts to come up with a simple way to express the amount of loss usually estimate it at about 0.5% to 1% per month (291, 292, 295, 296), roughly similar to postmenopausal bone loss rates over a year. Bone mineral density losses at landing after 6-month ISS missions are estimated to be 2% to 9% for different bone sites (297), with significant site-to-site and individual-to-individual variability. The bone loss and mineral shedding are accompanied by an increased risk of renal stone formation during and after flight (88, 89, 270, 298, 299). The subject-to-subject variability seems a characteristic of space flight-induced bone loss (300), and as more data are accrued, may provide insight to find a means to mitigate this loss (301).

Long-term follow-up data on bone recovery are far from complete (302, 303). However, if the rate of postflight recovery estimated from biochemical data is also assumed to be constant (reasonable according to ground-based (304) and flight (20, 55) data), then the rate of recovery is about +100 mg/d (20, 55). By these estimates, on flights up to about

6 months, it takes 2 to 3 times the mission duration to recover the lost bone. Analysis of bone recovery data from dual-energy x-ray absorptiometry (DXA) analyses suggests that while regional differences exist, the half-life of bone recovery after flight is on the order of 5-9 months after flight (301, 302). For longer exploration missions, however, the usefulness of these assumptions comes into question, as space flight data are not available for these durations. Although more data clearly are required to validate this hypothesis of bone mineral recovery, it nevertheless has significant implications as mission durations increase. Beyond bone mineral density, questions of changes to, and recovery of, bone architecture and strength also remain unanswered.

Bone Metabolism

Bone is a metabolically active tissue, constantly undergoing turnover through breakdown (resorption) and formation processes. When these 2 processes are in balance, no net loss (or gain) of bone occurs. Alterations in either, of both, of these processes can be problematic.

Bone resorption increases during space flight. This wasn't clearly documented until the 1990s, when markers specific to resorption were identified and analytical capability was commercialized. Collagen crosslinks are chemical linkages that give collagen its strength. These were found to be released during the resorption process and not metabolized before renal excretion. Many assay variants are available commercially, and are based on immunoassay techniques that bind to different portions of the molecules. Increased collagen crosslink excretion, and thus bone resorption, has been shown to be clearly increased during space flight (20, 55, 293, 305-308). Earlier studies showed that plasma concentrations of a similar but more easily confounded marker, hydroxyproline, were elevated during short-duration Shuttle flights (307) and longer duration Skylab flights (29, 160, 166). Calcium tracer kinetic studies also provided data indicating that bone resorption increases about 50% during flight relative to preflight (20, 55) levels.

Bone formation either remains unchanged or decreases during space flight (20, 55, 295). As indicated by serum concentrations of bone-specific alkaline phosphatase and osteocalcin, bone formation was unchanged during Mir flights, but increased 2 to 3 months after landing (20, 55). Trends toward decreased levels of bone formation markers were noted in 2 Mir studies with 1 subject each (293, 306). The results of studies, using calcium tracer techniques, of bone formation in 3 Mir crewmembers (20, 55) were equivocal (formation was unchanged or decreased). Together, increased resorption and decreased or unchanged formation yield an overall negative calcium balance (20, 55).

The exact triggering mechanism for these changes in bone metabolism during space flight has yet to be identified, but the physiological and endocrine responses to the changes are as expected. The release of calcium from bone suppresses parathyroid hormone (PTH), which results in lower levels of activated vitamin D (1,25-dihydroxyvitamin D), which leads to a reduction in calcium absorption from the gastrointestinal tract. Studies of calcium metabolism were conducted on Mir astronauts, and indeed, PTH, 1,25-dihydroxyvitamin D, and calcium absorption were all decreased (20, 55, 308). Although it remains important to maintain calcium intake during flight, the lower calcium absorption during flight suggests that increasing calcium intake is not a viable countermeasure for weightlessness-induced bone loss, a fact proven in bed rest studies (309, 310).

Space flight analog studies (such as bed rest) with humans have shown qualitative effects on bone and calcium homeostasis similar to those shown in flight studies (300, 311), with quantitative effects generally being of smaller magnitude. Effects

include loss of bone mass (304, 312-314), decreased calcium absorption (315), increased urinary excretion of calcium and biochemical markers of resorption (283, 314-326), increased risk of renal stone formation (87, 322, 326), and decreased serum concentrations of parathyroid hormone (281, 283, 316, 317, 319, 327) and 1,25 dihydroxyvitamin D (281, 315, 316, 319, 328).

That bone resorption increases during bed rest has been shown by histomorphometry (313, 329) and measurement of biochemical markers. Excretion of hydroxyproline (315, 323, 325) increases during bed rest, and excretion of collagen crosslinks (281, 283, 305, 314-317, 327) is elevated about 50% above control levels, compared with the increase of greater than 100% during space flight (20, 55, 305).

The concentrations of biochemical markers indicate that bone formation is unchanged during bed rest (281, 283, 312, 315-317, 327), but histomorphometry data from bone biopsies show that bone formation decreases (313, 319, 329). This difference likely reflects the difference between site-specific (biopsy) and systemic (biochemical markers) indices of bone formation. After ambulation begins following bed rest, bone formation generally increases (312, 315). Recent evidence indicates that with bed rest studies of longer duration (eg, 90 d), bone formation markers tend to increase (283, 314). In these initial publications, bone-specific alkaline phosphatase (BSAP} did not change significantly over time, but a retrospective analysis of data from multiple studies does show a change in BSAP that reaches statistical significance (Smith et al, unpublished observations).

Although early animal studies suggested that the primary change in bone metabolism was related to bone formation, the identification of markers specific to bone resorption in the late 1980s (330, 331) and the availability of commercial immunoassays in the 1990s (332, 333) allowed resolution of this matter: bone resorption increases during space flight and bone formation decreases or does not change significantly, substantiating that space flight disrupts the balance between bone resorption and formation, which can lead to a net loss in bone mass.

Bone Loss Countermeasures

Exercise

In-flight exercise was first implemented on Skylab missions, largely because this was the first vehicle to allow enough room for exercise. In addition to in-flight exercise, ground-based research has been conducted to help document the effectiveness of exercise as a countermeasure for muscle, bone, and cardiovascular maladaptations that occur during space flight (202, 311, 334, 335). With respect to bone specifically, treadmill and cycle exercise devices available on Mir did not prevent bone and calcium loss (20, 55, 292, 336, 337). This result has been attributed to the lack of resistance provided by these devices, and for the treadmill, the inability to generate sufficient ground-reaction force in weightlessness (338).

Bed rest studies evaluated many types of exercises and devices, alone or in rare cases in combination, demonstrating positive effects of treadmill, flywheel, and weight stacks on maintenance of bone (as assessed by various means from densitometry to biochemistry) (192, 279, 281, 339-343). Most evidence, from the general scientific literature as well as space flight analogs, pointed to resistance exercise as the most likely means of counteracting bone loss during space flight. Heavy resistance exercise in bed rest protected bone mineral density, but did so not by suppressing the bed rest-induced bone resorption, but rather by increasing bone formation (Figure 22).
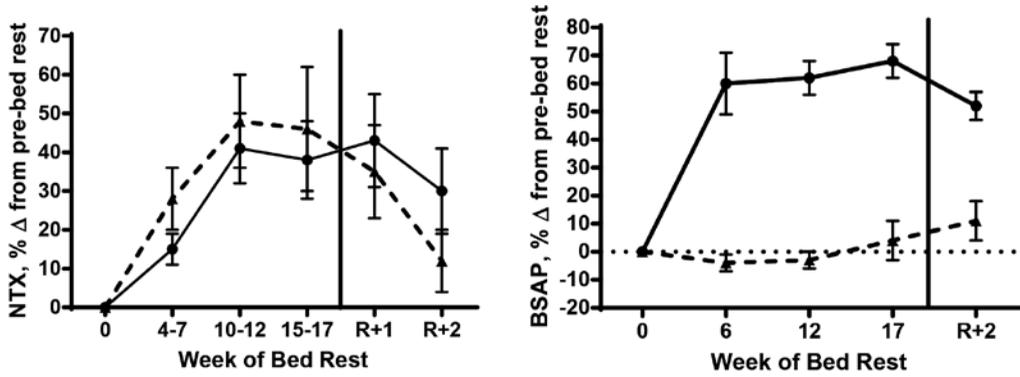
Figure 22. Bone resorption (as indicated by urinary n-telopeptide, left panel) and bone formation (as evaluated by serum bone-specific alkaline phosphatase [BSAP], right panel) during 17 weeks of bed rest with (solid line) or without (dashed line) heavy resistance exercise. Data are expressed as percentage of pre-bed rest values, and are mean ± SD. The vertical line separates the bed rest and post bed rest periods. Data adapted from (192).

Bed rest studies combining resistance exercise (with a flywheel device) and supine treadmill exercise while in a lower-body negative pressure (LBNP) chamber on alternating days yielded results similar to those shown above, but with about half the response of bone-specific alkaline phosphatase (317) (Figure 23).
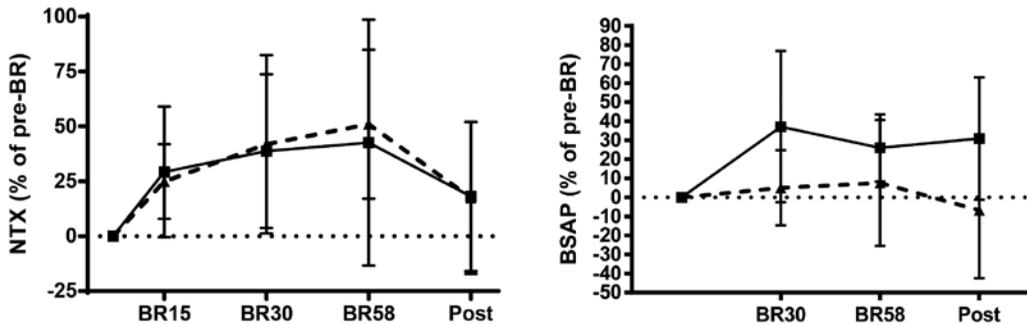

Figure 23. Bone resorption (as indicated by urinary n-telopeptide, left panel) and bone formation (as indicated by serum bone-specific alkaline phosphatase, right panel) during 60 days of bed rest with (solid line) or without (dashed line) a combination of resistance exercise and supine treadmill/ LBNP exercise. Data are expressed as percentage of pre-bed rest values, and are mean ± SD. Data adapted from (317).

Attempts to provide resistance exercise on early ISS missions were unsuccessful, with the interim resistance exercise device (iRED) providing no additional benefit over the Mir equipment (31, 294). This initial device was deployed on the inaugural ISS expedition, and time and other constraints did not permit development of all desired hardware requirements before this expedition was launched. Thus, the appropriately named interim resistance exercise device was to be used until a more advanced device capable of allowing heavier loads could be developed, tested, and launched to ISS.

Figure 24. Garrett Reisman shown using the interim Resistance Exercise Device (iRED, left), while Sandy Magnus is shown using the Advanced Resistance Exercise Device (ARED, right) on ISS. The iRED was launched in 2000 with the first ISS crew, and the ARED replaced it in late 2008.

In 2008, the Advanced Resistance Exercise Device (ARED) was launched to ISS. This device accommodated additional exercise protocols and had almost twice the loading capability of iRED (31, 344). Comparing crewmembers exercising with each device (Figure 24) has been somewhat difficult, given that more recent crews have maintained their energy intake at levels >90% of estimated requirements, and have had better vitamin D status than earlier crews. These better nourished crewmembers exercising with the ARED maintained body mass during flight (and came back leaner, with less body fat) (Figure 25), and as assessed by DXA (31, 345), maintained bone mineral density in most regions and in whole-body scans (Figure 26).
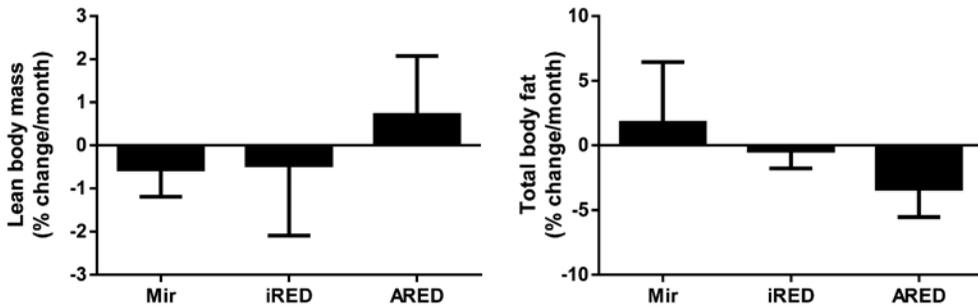

Figure 25. Body composition changes (left panel, lean body mass; right panel, total body fat) in astronauts on Mir and ISS missions. ISS crews had access to either iRED or ARED exercise devices. Data are expressed as percent change per month of flight and are mean ± SD. Figure adapted from (31).

Figure 26. Bone mineral density loss in astronauts on Mir and ISS missions. ISS crews had access to either iRED or ARED exercise devices. Data are expressed as percent change per month of flight. Figure adapted from (31).
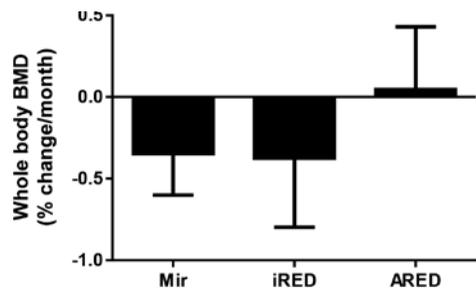
The number of ISS subjects whose data had been analyzed when the comparison of exercise devices was published in 2012 was small, in part because many crewmembers participated in other countermeasure studies and were not included in this initial analysis. In the time since that initial comparison of crewmembers with access to the iRED or the ARED was published, another study has confirmed these findings with a much larger set of data. In 42 astronauts (33 male, 9 female), the bone mineral density response to flight was the same for men and women (50) (Figure 27), and those with access to the ARED did not have the typical decrease in bone mineral density that was observed in early ISS crewmembers with access to the iRED (31). Biochemical markers of bone formation and resorption responded similarly in men and women. These recent data are encouraging, and represent the first in-flight evidence in the history of human space flight that diet and exercise can maintain bone mineral density on long-duration missions.

Figure 27. Bone mineral density (BMD) loss after flight in men (N=33, open bars) and women (N=9, solid bars) who used either the iRED or ARED exercise device. Data are expressed as percent change per month of flight and are mean ± SD. Figure adapted from (50).
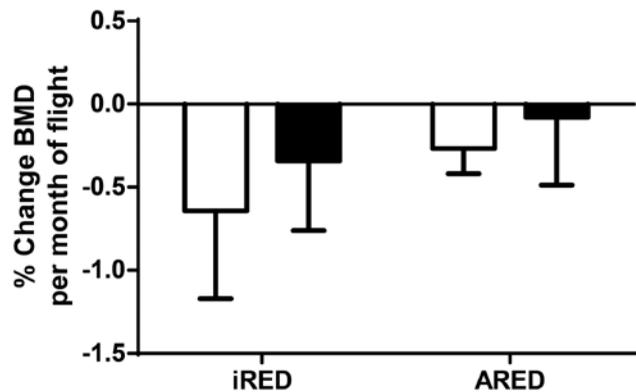
The bone biochemical changes in crewmembers exercising with the ARED were very similar to what had been observed in bed rest studies testing resistance exercise. That is, the exercise did not affect bone resorption, but did increase bone formation (31). In the flight study, as published, a significant ($P < 0.001$) increase occurred (31), and in bed rest, heavy resistance exercise 6 days a week led to dramatic increases in bone formation markers (192). In another study with resistance exercise every other day (combined with a treadmill protocol), subjects had roughly half the bone formation response (317) of subjects in the first study (192). The exercise also did not have a significant effect on serum total calcium or urinary calcium. When data from additional crewmembers became available, these solidified the "trend" into statistical significance (50). The slow increase in bone resorption over time during flight is likely related to the fact that the astronaut conditioning and strength trainers were initially reluctant to have crewmembers exercise too hard with the ARED, to minimize the risk of injury. This slow and steady increase over time (31) is different from results of the bed rest study, where formation markers plateaued at the first determination during bed rest (6 weeks of bed rest) (192).

Although this mode of bone remodeling, with increases in biochemical markers of both resorption and formation, maintained bone mineral density, it may yield a bone with strength characteristics different from those that existed before space flight. Studies to assess bone strength after flight are underway at NASA, to better understand the results of bone remodeling. Studies are also underway to evaluate optimized exercise protocols and nutritional countermeasures.

Gravity

Other physical countermeasures, including artificial gravity and vibration, have also been tested in ground-based (bed rest) settings. A more recent and complex study used centrifugation to create artificial gravity transients during a 21-day bed rest study (346). One hour per day of 1 G_z exposure at the level of the heart and 2.5 G_z at the feet was beneficial for some systems (eg, cardiovascular, muscle) (177, 346, 347), but this regimen did not have any effect on bone or calcium metabolism (327, 348). Although greater durations, increased *g* forces, or combining centrifugations with exercise protocols have been proposed, these have not yet been extensively tested (346, 349).

The assumption that it is lack of gravity that stimulates bone loss during space flight provided the impetus for proposing replacement of gravity by centrifugation ("artificial gravity") as a countermeasure for multiple body systems (350, 351), particularly for bone. The statement has been made that it is known that unit gravity maintains bone, whereas microgravity does not. A key question is, "How much gravity do we need?" (W. Paloski, personal communication). In early bed rest studies, 2- or 4-hour intervals of standing or walking mitigated the increase in urinary calcium excretion associated with bed rest (352). Some of the artificial-gravity studies have relied on short-radius centrifuges (353), others on rotating exercise devices (349, 354) intended to provide gravitational impact as well as physical exercise. Artificial gravity or hypergravity has been shown to positively affect bone, in human and some animal studies (355-357). As noted above, 1 hour per day of centrifugation resulting in 1 G_z at the heart and 2.5 G_z at the feet was ineffective for bone (327). The optimal artificial gravity prescription for bone, including dose, duration, and frequency of centrifugation, remains to be clarified (358), along with its potential impact on nutrition and related systems (359).

Vibration

Protocols for exposure to vibration, of both high and low frequency, have also been proposed and tested in space flight analogs. Although low-frequency protocols showed promise for protecting bone in both animal and ambulatory human studies (360-362), the beneficial findings were more limited when testing occurred during head-down-tilt bed rest (363). Higher frequency vibration, often referred to as resistance vibration exercise, has generally shown positive effects on bone and muscle during bed rest (206, 364-366). Debate continues over the potential of this countermeasure protocol, amid safety concerns about neuromuscular issues with repeated vibration exposures.

Pharmacological Agents

Pharmacological agents, the most common being the bisphosphonates, have also been tested for their ability to mitigate weightlessness-induced bone loss. Many ground analog studies of bisphosphonates (including bed rest studies and studies of patients immobilized because of spinal cord injury or other reasons) have been conducted, with generally positive findings (313, 335, 340-342, 367-374). However, ongoing discussion and debate surround the relative safety of these compounds for use in otherwise healthy individuals (astronauts), as opposed to the target population for whom the drugs were developed (patients with disorders such as osteoporosis). In addition to resolving safety concerns, investigators have yet to determine the optimal drug, dose, and schedule of administration during space flight. As noted above with exercise, given that the bone loss of bed rest is about half that of space flight, there is little reason to believe that the same dose of drug will have the same effectiveness in both environments.

Moreover, data from animal studies suggest that the disuse- or space flight-induced increase in bone resorption cannot fully, or chronically, be mitigated by bisphosphonates (375, 376).

Endocrine therapies, including exogenous calcitonin administration (325, 335), have also been attempted, albeit unsuccessfully. In animal models, testosterone has also been suggested as a bone loss countermeasure (221, 222) on the basis of limited data showing a reduction in testosterone concentrations during flight in human, animal, and cellular models (223-229). Reduction of testosterone, however, has recently been shown to likely not be a concern during space flight. See section 3, "Muscle and Protein," for a more detailed discussion of these data.

Nutritional Countermeasures

One of the most obvious nutritional countermeasures—providing calcium—does not protect against bone loss (377). This result is likely related to the decreased calcium absorption seen during bed rest (315, 348) and space flight (20, 55, 308), likely related to reduced circulating parathyroid hormone and 1,25-dihydroxyvitamin D. Phosphate supplementation, used in an attempt to reduce calcium excretion, was also ineffective (379). Combination therapy with calcium and phosphorus was also unsuccessful at mitigating bone loss and hypercalciuria (325).

Omega-3 fatty acids have been shown to protect bone, in the general population (113-115) and animal studies (380), as well as in space flight analog studies, including bed rest (Figure 28) and cell culture (116). While omega-3 fatty acids have not been studied in a controlled fashion during actual space flight, a positive correlation was found between fish intake and bone loss in astronauts (Figure 28) (116). That is, those who ate more fish lost less bone. These data provide additional evidence of the potential importance of fish oils as a countermeasure for muscle, bone, and radiation risks of space flight. Studies showing positive effects of omega-3 fatty acids typically look at intake of fish or other food sources of these nutrients (117-119). Studies of fish oil supplements added to typical diets often fail to document any benefit (120-122), thus highlighting the need for dietary modification, and not simply supplementation.
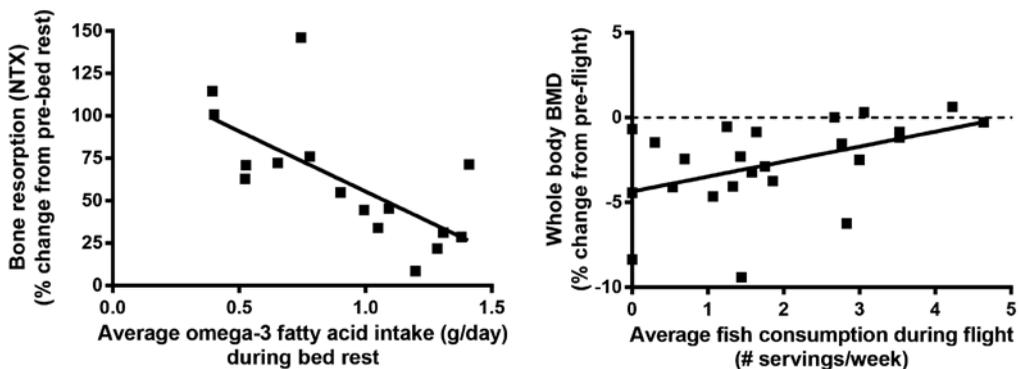

Figure 28. Relationship between omega-3 fatty acid consumption and bone resorption during bed rest (left panel), and relationship between fish consumption and percent change in whole-body bone mineral density at landing in astronauts (right panel). Figures adapted from (116).

Other nutrients, specifically sodium, protein, potassium, and vitamin K, have been documented to have effects on bone, and/or have been proposed or tested as countermeasures to bone loss (280). These are discussed in detail in other sections of this book.

Nutrients and Bone Health

Calcium

Calcium metabolism is of critical importance in bone health, and many studies use calcium determinations as one measure of bone health; however, it has been well documented that excess dietary calcium will not mitigate bone loss during space flight (310, 381). Nonetheless, ensuring adequate calcium intake remains important, and the role of calcium excretion in kidney stone risk must be considered. Two other facets of calcium chemistry and biochemistry have been researched, with intriguing findings. One is related to the impact of calcium in the reclamation of water from urine on spacecraft, and the other relates to naturally occurring stable isotopes of calcium and their ability to document changes in bone metabolism.

Calcium Balance

Negative calcium balance was observed during the Skylab (29, 160, 288, 321, 382-384) and Mir (20, 55) missions. During the 84-day Skylab 4 mission, calcium balance was –200 mg/d (160, 384, 385). Increased urinary and fecal calcium excretion accounts for most of the calcium deficit (20, 29, 55, 160, 288, 299, 321, 383). Estimates of bone calcium loss from multiple studies and techniques converge on the suggestion that about 250 mg of bone calcium is lost per day during flight (20, 55, 160, 386). When this rate of loss may slow down is not yet known, but it does not appear to be within the first 6 months of flight. For comparison, bone loss after spinal cord injury seems to stabilize after about 6 to 12 months (387-389), around the duration of many ISS missions.

Vitamin D

The best-understood role of vitamin D is its involvement in calcium metabolism. One of the major functions of this vitamin is to maintain normal blood levels of calcium and phosphorus. The liver converts vitamin D to 25-hydroxyvitamin D. Typically the gold-standard measurement for determining vitamin D status, 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitamin D in the kidney, and from there it is transported systemically to target organs. Classic target organs include bone, intestine, and kidney.

The Institute of Medicine (IOM) conducted an extensive review of the literature in 2010 and increased the recommended dietary allowance (RDA) for vitamin D to 600 IU/d for healthy males and females 9-70 years old, and 800 IU/d for those older than 70 years (390). The main factors that were taken into account were changes in bone density and fracture risk. The IOM Committee did not find conclusive evidence that vitamin D plays a role in extraskeletal health outcomes. Even though a considerable number of papers published in the past 10 to 15 years have shown associations between vitamin D status and adverse health outcomes such as cancer, autoimmune diseases, diabetes, cognitive decline, and all-cause mortality, the evidence that vitamin D status causes any of these outcomes is inconclusive, and insufficient to inform nutritional recommendations (391-396). The noncalcitropic functions of vitamin D may help explain why a robust set of reproducible data shows an inverse correlation between sun exposure and

several types of cancer (397-399). Although 1,25-dihydroxyvitamin D is the biologically active form for the noncalcitropic functions also, 25-hydroxyvitamin D must be available in sufficient quantities for the 1-hydroxylase enzyme in nonrenal tissues to synthesize 1,25-dihydroxyvitamin D. Besides kidney cells, other cell types, including bone cells, epithelial cells, monocytes, and antigen-presenting cells, also synthesize 1,25-dihydroxyvitamin D (400). Numerous tissues are affected by vitamin D status because their cell nuclei contain receptors for 1,25-dihydroxyvitamin D (394). Some of these tissues are adipose tissue, bone marrow, brain, breast, cancer cells, cartilage, lung, muscle, ovary, placenta, prostate, stomach, testis, thymus, and uterus (401).

People who are normally exposed to sunlight make vitamin D in their skin. Ultraviolet B light, a component of sunlight, converts 7-dehydrocholesterol to 25-hydroxyvitamin D_3 in the skin (402). Although sunlight has a positive effect on health through its role in making vitamin D, caution must still be exercised to avoid too much sun exposure (403-405).

Before 2006, when ISS crews were first provided 400 IU vitamin D/d, it was well documented that vitamin D status (serum 25-hydroxyvitamin D) decreased after long-duration space flight (6, 20, 29, 55). The absence of ultraviolet light during space flight diminishes vitamin D stores in the body, as observed during the 84-day Skylab mission (29), space station Mir missions (20, 55), and early ISS expeditions (6). Despite the reported use of vitamin D supplements by some of the astronauts on early ISS expeditions (average supplement use was 3.0 ± 2.8 per week of a 400-IU vitamin D supplement), the mean serum concentration of 25-hydroxyvitamin D for the ISS crewmembers was about 25% less after landing than before launch.

In 2006, vitamin D recommendations to crews increased from 400 IU vitamin D/d to 800 IU vitamin D /d. In-flight 25-hydroxyvitamin D data from the Nutritional Status Assessment Supplemental Medical Objective provide evidence that 800 IU vitamin D/d is enough to maintain vitamin D status during long-duration space flight (Figure 29) (31). Furthermore, recent data show that crewmembers who exercised with the ARED, maintained energy intake at recommended levels, and took 800 IU/d vitamin D during flight maintained bone mineral density during 4- to 6-month space flights (31).

An ideal ground-based model for individuals lacking ultraviolet light exposure is the Antarctic, where winter levels of ultraviolet B radiation are essentially zero. Two studies have been conducted at McMurdo Station, Antarctica, to determine the dose of vitamin D needed to sustain serum levels of 25-hydroxyvitamin D during a 5- to 6-month period when there is little to no ultraviolet B exposure, without increasing risks of hypercalcemia (406, 407).

Several ground-based studies (performed in Antarctica and at the Johnson Space Center) provide evidence that a vitamin D dose in the range of 800-2000 IU/d is tolerable and safe, and can maintain vitamin D status for 3 to 6 months even in environments with no UV light exposure (406-408). This recommendation is in line with the recent Institute of Medicine recommendations for vitamin D intake for North Americans (390). One study conducted at McMurdo Station in Antarctica showed an interactive effect of serum cortisol and vitamin D status on immune function (407). In that study, subjects with higher serum cortisol and lower vitamin D status presented with more latent virus reactivation in their saliva. It is clear that vitamin D may have an effect on other systems besides bone, but further research is required before evidence-based recommendations can be made for other systems.

Figure 29. Pre- and postflight data (mean ± SD) from testing required for medical operations show that vitamin D status decreased after long-duration space flight, despite vitamin D supplementation with 400 IU/d (black dashed line, N=16). In-flight data (purple line) show that 800 IU/d is enough vitamin D_3 to maintain status during long-duration space flight (N=26). Red lines depict Institute of Medicine-defined lower acceptable limits (with respect to bone health), and upper advised limit (390). The green line at 80 nmol/L reflects what many perceive as an optimal level with respect to parathyroid hormone suppression and non-bone health outcomes. Figure adapted, and data updated, from Smith et al (409).
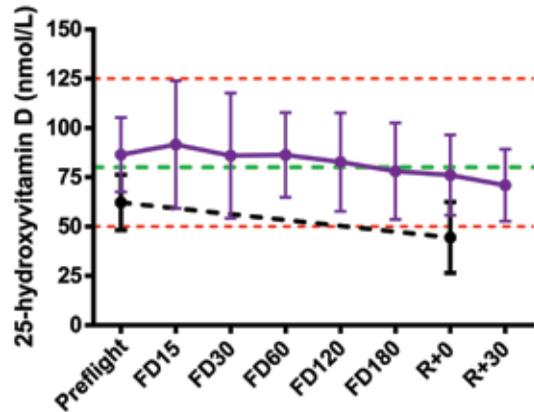
As noted above, vitamin D deficiency is linked to calcium metabolism, and in severe cases leads to osteomalacia and osteoporosis in adults (and rickets in children). Throughout the ISS program, supplemental vitamin D has been provided to astronauts to ensure optimal vitamin D status.

Efforts to provide vitamin D supplements are misinterpreted to infer that this might be a viable bone loss countermeasure, but this is not the case. Even when vitamin D stores during flight are adequate, the circulating concentration of the active form of vitamin D, 1,25-dihydroxyvitamin D, is decreased (20, 55). As described in the previous subsection, "Calcium," this is likely the result of the increased release of calcium from resorbed bone, and results in decreased intestinal absorption of calcium. Adequate stores of 25-hydroxyvitamin D will not affect this. Any attempt to directly provide the 1,25-dihydroxyvitamin D, or as in some cases on Earth, excess 25-hydroxyvitamin D levels, may lead to hypercalcemia, renal stones, soft-tissue calcification, and even death. Controlled trials in bedridden subjects have also proven that several months of supplementation fail to affect bone metabolism. In one trial, bedridden elderly people took supplemental vitamin D (400 or 1200 IU/d compared to placebo) for 6 months. Little effect was found on parathyroid hormone, and no effect on bone markers (410). In a similar 40-week trial of 1000 IU of vitamin D_2 or D_3 (2 groups), neither had an effect on bone markers (309). The problem of weightlessness-induced bone loss must be solved, but vitamin D is not the answer. Nevertheless, even if bone loss is not stemmed, ensuring an adequate amount of vitamin D will remain important.

As the current space food system includes very few dietary sources of vitamin D, and vitamin D cannot be synthesized endogenously due to lack of UV light, decreased vitamin D status is a serious concern for exploration missions that could last 1000 days.

Toxicity of vitamin D is typically less likely to occur than a deficiency (411-414), but use of supplements would increase its likelihood. Excessive blood levels of vitamin D can lead to hypercalcemia, which can lead to nephrocalcinosis, arteriosclerosis, and irreversible soft tissue calcification. In one study conducted in Houston in healthy individuals, a 50,000 IU/wk dose of vitamin D for 4 weeks and then monthly increased mean urinary calcium excretion to numbers higher than the normal range (408). In that study, a daily dose of 2000 IU or a single weekly dose of 10000 IU did not increase the incidence of hypercalciuria.

Vitamin K

The function of vitamin K was originally assumed to be strictly limited to involvement in blood coagulation, but an increasing amount of evidence indicates that this vitamin affects multiple physiological systems. Vitamin K is a cofactor in the posttranslational synthesis of γ-carboxyglutamic acid (GLA). γ-Carboxyglutamic acid is a constituent of all vitamin K-dependent proteins, and its role is related to increasing the affinity of the proteins for calcium (415). Vitamin K-dependent proteins include blood coagulation proteins and bone proteins (eg, osteocalcin, matrix GLA protein, protein S). On the basis of findings from Mir in the 1990s and from early ISS missions (described below), vitamin K had been proposed as a bone countermeasure (416).

Data from 11 US astronauts on ISS Expeditions 1 to 8 (mission durations of 128 to 195 d during 2000-2004) revealed that on landing day their serum phylloquinone (vitamin K_1) was 42% lower than it was before flight, whereas urinary GLA did not change (6). In another study, undercarboxylated osteocalcin was elevated (a sign of vitamin K insufficiency) as early as the eighth day of space flight, and remained high during 21- and 180-day missions (417). Studies on the EuroMir 95 mission showed that markers of vitamin K status were decreased after 12.5 weeks of space flight, and vitamin K supplementation (10 mg/d for 6 wk) reversed these effects (418). Vitamin K supplementation elevated GLA and decreased undercarboxylated osteocalcin, suggesting that vitamin K status was lower during space flight and was improved by supplementation (417, 418). Despite the changes on landing day, the monitoring of vitamin K status during flight has documented no evidence that vitamin K status is decreased during space flight. In-flight data from 15 crewmembers on Expeditions 14 to 22 showed no major changes in phylloquinone, urinary GLA, or undercarboxylated osteocalcin (419). Phylloquinone data from those 15 crewmembers (419) plus an additional 11 crewmembers on Expeditions 23 through 31 are shown in Figure 30. Even with the additional data, vitamin K status did not significantly decrease during flight. This is an important finding, to document that a vitamin K countermeasure to mitigate a vitamin K deficiency during space flight is not needed and therefore likely would not have an effect on bone.
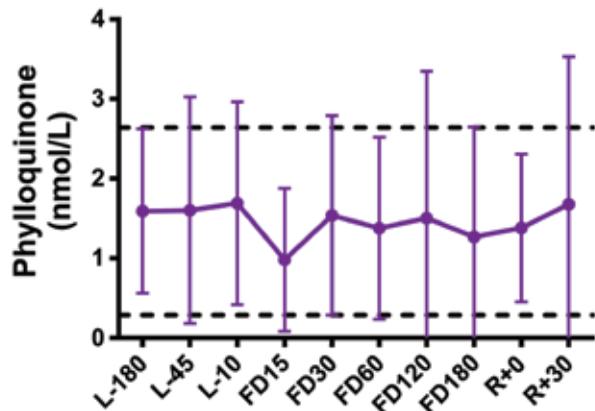

Figure 30. Serum phylloquinone before launch (launch minus 180 d, or L-180, L-45, and L-10), during flight (flight days 15, 30, 60, 120, and 180), on landing day (R+0), and 30 days post landing (R+30). The dashed lines indicate the normal range for phylloquinone. Data are mean ± SD, N=26. Data and N are expanded from the original publication of these findings (419).

Phosphorus

Phosphorus is an important component of cell membranes and bone mineral. Phosphate accounts for about 60% of bone mineral (420), and most (85%) of the body's extracellular phosphorus is in bone (421). After ISS missions, urinary phosphorus excretion was about 45% lower than preflight excretion (6).

Excretion of phosphorus during untreated bed rest was not changed (283) from ambulatory conditions. An earlier study of 3 subjects revealed increased urinary phosphorus and negative phosphorus balance (323). In bed rest studies, investigators have attempted to use combination therapy with calcium and phosphorus to mitigate bone loss and hypercalciuria, with trends in the right direction but no significant changes (325).

High phosphorus intake, relative to calcium in particular, can have effects on many systems, including bone, kidney, and cardiovascular (422-424). Ideally, the ratio of calcium:phosphorus should be around 1.0, or higher. The ISS requirements match this, with a notation that the phosphorus:calcium ratio should not exceed 1.5 (2, 425, 426), based on evidence that a dietary phosphorus:calcium ratio greater than 1.5 is known to decrease calcium absorption, which could further impair skeletal integrity. Serum phosphorus rises with increasing phosphorus intake, and if hyperphosphatemia occurs, it can result in calcification of the kidney. For this reason, ensuring optimal phosphorus intake during flight becomes very important (295). To date, phosphorus intakes have been higher than desired. The standard menu has a P:Ca ratio of 1.8 (2); actual intakes have been slightly lower than that, but still greater than 1.5 (Figure 31). Bed rest studies have tended to have P:Ca ratios closer to 1.0 (unpublished observations).
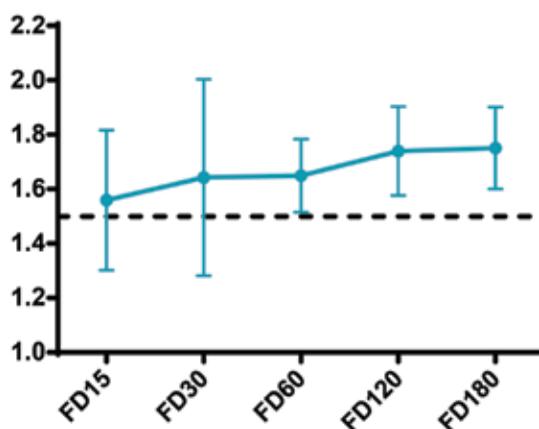

Figure 31. Dietary phosphorus:calcium ratio in ISS crewmembers during flight (approximate flight days 15, 30, 60, 120, and 180). The dashed line indicates a ratio of 1.5. Data are mean ± SD, N=9.

Magnesium

Magnesium is the fourth most abundant cation in the body, and more than half of the body's magnesium is in bone (427). Adequate intake of magnesium is necessary to prevent hypocalcemia, resistance to vitamin D, and resistance to parathyroid hormone. Excessive magnesium intake from supplements has been shown to impair calcium absorption (427).

Decreased urinary magnesium after flight, relative to before flight, seems to be a hallmark of space flight (29, 428). Serum magnesium trends downward during and after flight, as seen with Skylab and Shuttle crews (2, 6, 29, 429, 430). After crewmembers had spent 4 to 6 months in space on ISS, their urinary magnesium was about 45% less than it was before flight (6).

Autopsy results after the tragic end of the 24-day Salyut-1 mission documented that, relative to control subjects, the Salyut-1 cosmonauts had 12% to 32% lower concentrations of magnesium in the compact layer of the femoral epiphysis and diaphysis, vertebral body, and sternum (431). These changes were reported as appearing "with a high degree of certainty." Magnesium balance was slightly negative during extended-duration bed rest studies conducted in Russia (341), with little effect

of exercise or bisphosphonate. Recent studies conducted in the US have shown a decrease in magnesium excretion in short- and long-duration bed rest (134, 283).

Magnesium shows promise for reducing the risk of renal stone formation (432). In ground-based studies, potassium-magnesium citrate has proven effective in reducing bed rest-induced risk (87). Potassium citrate (KCit) has been successfully tested during ISS missions (433), and has been "transitioned to operations." This means that KCit is part of the flight surgeon's toolbox for helping crewmembers mitigate renal stone risk. That is, it is available on ISS for use based on flight surgeon discretion if clinically indicated. However, given that fluid intake for optimum hydration is a preferred countermeasure, and some residual concerns exist about side effects of potassium supplementation, it was decided not to routinely provide KCit to crewmembers.

Zinc (and Lead)

Zinc status of astronauts, as assessed by serum zinc and urinary zinc excretion, did not change after long-duration space flight (6). Circulating zinc levels are an imperfect tool to evaluate zinc status, as other physiological factors may affect them (434). However, to increase the reliability of zinc status evaluation, more intensive and/or invasive techniques would be required.

The release of zinc from bones (as a result of demineralization) has been noted in bed rest studies (435, 436), and a similar increase in excretion of zinc was noted in Wistar rats flown during COSMOS 1129 (a 20-d space flight) (437). This release of zinc associated with demineralization has raised concern that other metals, including lead, could also be released secondary to weightlessness-induced bone resorption (438, 439). Garcia et al developed a computer-based model that predicted that blood lead levels would actually decrease during microgravity exposure. The model predicted, for the majority of astronauts, that any increase in circulating lead would be more than offset by decreases in ingested or inhaled lead during the mission (440). Postflight data supported this model (440).

Unique Aspects of Calcium and Space Flight

Urine Processing and Water Reclamation

The ability to reclaim water from urine will be a pivotal factor in implementing exploration-class missions. When the prototype Urine Processor Assembly (UPA) on ISS clogged due to an unknown precipitate in 2009, the available in-flight urine volume and calcium excretion data from 24-hour pools and single voids were closely examined (441). Urine 24-hour volume was about 17% lower during flight than before flight, and urinary calcium concentration was 50% greater during flight than before flight. The increased urinary calcium concentration during flight was identified as a primary reason for UPA failure, and new recommendations for percentage of water to be recovered were made as a result of those findings. In 2012, when the data were re-evaluated with data from an additional 10 subjects in a Supplemental Medical Objective study of nutritional status assessment, it was clear that crewmembers in recent years are drinking more fluid than crewmembers did in the past, and as a result urinary calcium concentration is lower. The UPA extracts water by vacuum distillation, and as a result, the primary concern for the UPA is the concentration of calcium in the urine, not the amount of calcium excreted (441). Suggestions for ways to mitigate high calcium concentrations included exclusion of the first morning void from the system, based on the assumption that this

is typically more concentrated than other voids throughout the day. Another suggestion was administration of bisphosphonates to all crewmembers to reduce calcium excretion. Calcium concentration data from the in-flight single-void data showed that neither of these suggestions looked promising for lowering the calcium concentration of urine that enters the UPA (441). Regarding bisphosphonate treatment, people sometimes have the impression that the pharmacological blocking of bone resorption will reduce urine calcium to near zero, but this is not the case. In fact, although urinary calcium decreased in ISS crewmembers treated with bisphosphonate, it had started higher in these individuals, so the daily excretion was about the same as for individuals who did not receive bisphosphonate (345).

When in-flight urinary calcium concentration was examined in light of in-flight mean fluid intake estimated from the food frequency questionnaire, all but one 24-hour pool had calcium concentrations below 23.7 mg/dL (the cutoff point for a 75% water recovery) when fluid consumption was greater than 32 mL fluid/kg body weight. For the 23 crewmembers in that analysis, that would average to a fluid consumption (from food and beverages) of 2.5 L/d. In September 2012, a decision was made to increase recovery from 70% to 74%, which allows a savings of >80 L H_2O/year.

Natural Calcium Isotope Composition of Bone

Analytical techniques to assess bone health, bone loss, and bone metabolism continue to evolve with technology. Although densitometry techniques (such as DXA and quantitative computerized tomography) provide valuable assessment of specific bones, these techniques detect only relatively large changes in bone, which can take months to occur, despite the likelihood that biochemical changes are initiated within hours of exposure to space flight. Studying calcium metabolism requires either intensive balance studies or tracer kinetic studies, as calcium excretion alone is confounded by too many factors to be useful in non-controlled studies. Bone formation and resorption markers provide the ability to assess changes in bone biochemistry, but assessing the relative association of these 2 factors has not been possible to date, and thus it is difficult to assess net changes in bone mineral.

A new technique to rapidly detect and quantitatively predict changes in whole-body bone mineral balance was recently validated in bed rest (442). This technique is based on natural, biologically induced variations in the presence of the naturally occurring stable (nonradioactive) calcium isotopes (^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca, and ^{48}Ca), which react at different rates depending on their mass (443). These variations exist because bone formation favors the lighter isotopes, and thus the blood and urine (and other "soft tissue") tends to have the "heavier" calcium isotopes. Bone resorption, however, releases whatever calcium is in bone, and that tends to be the lighter isotopes. Thus, when bone is being resorbed (as it is during bed rest), the urine contains greater amounts of the lighter calcium isotopes than it did before the increased resorption. When the isotope ratio technique was applied to a bed rest study, it was shown that the calcium isotope ratio shifted in a direction consistent with bone loss after just 7 days of bed rest, long before detectable changes in bone density occur. Consistent with this interpretation, the calcium isotope variation accompanied changes observed in n-telopeptide, whereas bone-specific alkaline phosphatase, a bone-formation biomarker, was unchanged (442).

As the relationship between calcium isotopes and whole-body bone mineral balance is well established (444-446), this relationship can be used to quantitatively translate the changes in the calcium isotope ratio in urine to changes in bone mineral density using

a simple model. Using this model it was estimated that subjects lost 0.25 ± 0.07% (1 SD) of their bone mass from day 7 to day 30 of bed rest (442). This rate of loss extrapolates to a loss of 1.36 ± 0.38% of skeletal mass over 119 days, which is equivalent, within error, to bone loss rates determined by DXA scans in long-term (119-d) bed rest studies (314).

Given the rapidity with which calcium isotope measurements detect change and their potential for use in assessing bone loss, this technique is ideally suited for space flight studies in which changes in bone formation and resorption are not only being altered by space flight itself but are being manipulated by various countermeasures.

ISS flight control team celebrating and toasting the first drink of the on-board water reclamation system on the space station during Expedition 19.

5 Iron and Hematology

Iron

Iron is an essential element involved in oxygen transport, oxidative phosphorylation in carbohydrate and lipid metabolism, and electron transport in cytochromes and cytochrome oxidase (447, 448). Adequate iron is crucial for meeting the needs of organs and tissues, but excess iron is detrimental to cells and can cause oxidative damage. The body achieves iron balance through regulation of absorption by enterocytes in the intestine and regulation of iron export from cells. Once iron is absorbed into the enterocyte, it can be bound to ferritin and stored. Serum ferritin has been shown to be a sensitive indicator of iron stores (449). Ferritin is exponentially correlated with storage iron, as determined by quantitative phlebotomy in patients with iron overload (450).

Iron deficiency is the most common nutritional deficiency worldwide, but iron toxicity is also worthy of concern. Deficiency of iron leads to anemia, fatigue, reduced work capacity, impaired behavior and intellectual performance, cognitive deficits and memory loss, heart palpitations, impaired thermoregulation, and decreased immune function (451-453). Toxicity of iron may lead to tissue damage or cancer. High iron intakes have also been related to gastrointestinal distress. The toxic potential of iron derives from its ability to exist in 2 oxidative states (ferrous and ferric forms). Iron serves as a catalyst in redox reactions; however, when these reactions are not properly modulated by antioxidants or iron-binding proteins, cellular damage can occur (454). Adaptation of iron metabolism in humans typically allows the maintenance of normal body iron concentrations in spite of disparate physiological requirements and dietary supply (455). Body iron, about 4 g in the adult human, is determined by physiological iron demands, dietary supply, and adaptation (447, 455, 456). Dietary iron is a function of both content and bioavailability of total food iron; bioavailability is lower in non-heme than in heme iron sources. Dietary factors that inhibit iron absorption include tea, coffee, bran, calcium, phosphate, egg yolk, polyphenols, and certain forms of dietary fiber (447). Conversely, meat, fish, poultry, and ascorbic acid will enhance the bioavailability of non-heme iron.

Iron is known to be involved in immune system function—specifically, adaptive and innate immune response—and both iron overload and iron deficiency affect immune function. As reviewed by Dao and Meydani, iron overload can affect susceptibility to infection (457). On the other hand, iron deficiency affects the function of certain immune cells, including neutrophils and natural killer cells, and production of cytokines (457). As with any nutrient, supplementation must be used with caution, as some have found that in areas of the world where infection rates are high, such as malaria-endemic regions, iron supplementation can actually increase risk of infection, suggesting that the supplemented iron provides an environment for pathogens to thrive (458). Others have shown that iron deficiency can help protect against some types of infections (459).

Evidence from short- (weeks) and long-duration (months) space missions shows that red blood cell (RBC) mass decreases during flight because of neocytolysis (460, 461). An early hypothesis for the cause of decreased RBC mass was that RBC synthesis

in space was understimulated relative to synthesis on the ground (462). Decreased release of mature RBCs into the circulation is associated with a decrease in circulating erythropoietin concentrations. Serum erythropoietin decreases in the first few days of space flight, but it returns to preflight levels later and iron turnover is unchanged during flight (461, 463), indicating that synthesis of RBCs and hemoglobin is unchanged. A consequence of the decreased red blood cell mass is the subsequent transfer of the iron from newly synthesized cells into storage proteins and processes. Evidence of this includes increased circulating concentrations of serum ferritin, an index of iron storage, after short- and long-duration space flights (6, 430, 464). In addition to these physiologic changes that can affect tissue iron stores, dietary iron content is very high in the International Space Station (ISS) food system, largely because many of the commercial food items in the ISS menu are fortified with iron (2). The mean iron content of the standard ISS menu is 20 ± 6 mg/d, and individual crewmembers have had intakes in excess of 47 mg/d for some weeks during long-duration missions. For reference, the defined space flight requirement for iron is 8-10 mg/d for both men and women (2, 425), and the current US Dietary Reference Intake (DRI) for men is 8 mg/d and the DRI for women is 10 mg/d (465). The tolerable upper intake limit for iron as defined by the Institute of Medicine is 45 mg/d (465).

Indices of iron metabolism and erythropoiesis return toward normal relatively quickly (days) after landing, although the replenishment of RBC mass may take several weeks. The repletion of RBCs usually occurs after the disproportionate return of plasma volume, so that a dilutional "anemia" often occurs after flight (466). For example, a 3% to 5% decrease in hematocrit between landing (R+0 d) and R+3 days is common after both short- and long-duration flights (466).

Although the in-flight decrease in RBC mass is substantial, the efficient postflight recovery suggests that the change represents an adaptation to weightlessness. After the first weeks of flight, RBC mass and body fluid volumes reach new plateaus (lower than on Earth), as shown by data from long-duration flights (35, 428, 467, 468). The triggering mechanism for these changes is unknown. One hypothesis is that the body senses a decreased requirement for blood volume and adapts accordingly. This may be related to changes in fluid (circulatory) dynamics and reduced gravitational strain on the circulatory system during flight, which may result in easier delivery of oxygen to tissues, or to the decreased plasma volume and increased concentration of RBCs in the first few days of space flight. The decrease in RBC mass has no documented functional consequences.

In-flight data show that iron stores increase early during a mission (within 15 d) and then return to preflight concentrations by the end of a 6-month mission (469). In a recent study with 23 crewmembers of missions 50 to 247 days in duration, ferritin increased about 220% in women and 70% in men by flight day 15 (469). At several time points, the transferrin index exceeded 1 µmol iron/µmol transferrin, which provides evidence that iron overload occurred (470). Other acute-phase proteins (C-reactive protein and ceruloplasmin) were not changed during flight, indicating that the ferritin response was likely not just an inflammatory response. In this study the amount of increase in ferritin (area under the curve) was associated with the change in bone mineral density after flight, which was supported by the association between ferritin and other markers of iron status and markers of bone resorption. The greater the increase in ferritin during flight (or the longer it was elevated; either case would result in a greater area under the curve), the greater the decrease in bone mineral density in the hip, trochanter, hip neck, and pelvis after long-duration space flight (469). The change in ferritin over the course of

a 6-month mission is presented in Figure 32, and is very similar to the change in urinary 8-hydroxy-2'-deoxyguanosine (8OHdG, a marker for oxidative damage) during space flight). These data are important to show that mean ferritin concentrations during flight that were not outside the normal clinical range were associated with evidence of oxidative damage and bone resorption, and this is supported by other studies in healthy ground-based populations (471-473).

Bed rest studies have not proven to be consistently reliable models for the

Figure 32. The percent change from preflight (mean ± SD) in serum ferritin (red circles, solid line) and urinary 8OHdG (blue squares, dashed line) before, during, and after long-duration space flight (N=23). "Pre" was determined from the mean of preflight data points (3 for ferritin, 4 for 8OHdG), and percent change was calculated from that average. (469)

hematological changes of space flight. Early bed rest studies showed a decrease in RBC mass during bed rest, but erythropoietin was unchanged and hematocrit increased (474), suggesting that the mechanisms that bring about hematological changes during bed rest are different from those that act during flight. If the reduced RBC mass during flight is caused by the reduced gravitational load on the circulatory system, it is reasonable to assume that bed rest alone would not alleviate these forces, but would only reposition them. More recent studies have shown small changes in iron status measurements, the most consistent being a drop in hematocrit and hemoglobin after reambulation (134, 283), suggesting an impact of plasma volume replacement, with a smaller role of hematopoiesis.

Another model is provided in studies involving changes in altitude, where the descent from high to low altitude induces changes similar to those observed for space flight (decreased red cell mass, increased iron storage) (475). Exogenous erythropoietin prevented the changes (475), suggesting that it is involved in the regulating mechanism, as it may be in the initial change in space flight.

The NASA Extreme Environment Mission Operations (NEEMO) undersea environment provides an excellent space flight analog for changes in iron status, specifically with respect to the environment in the NEEMO habitat (476). Because of the increased air pressure in the habitat, crewmembers are exposed to higher oxygen pressures, which increase their risk for oxidative damage to DNA, proteins, and lipids (477-480). Probably because of the increased pressure and greater oxygen availability, body iron stores are elevated during the saturation dive (6, 464). Serum ferritin is routinely increased during the NEEMO saturation dives (10-14 d long), and evidence for oxidative damage and stress is also observed (476). On a recent NEEMO mission, red blood cell folate was decreased during the dive, and plasma folate status was inversely correlated with serum ferritin (481). Decreased superoxide dismutase activity and peripheral blood mononuclear cell poly(ADP-ribose) were also evident during the dive, indicating a DNA repair response occurred (481).
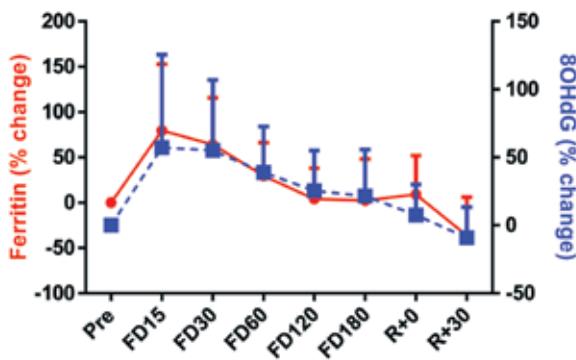
The implications of moderately increased iron stores in the body include exacerbated bone loss, oxidative stress, cardiovascular disease, and cataracts or other ophthalmic issues. For example, ground studies show that in healthy subjects, increased body iron stores (assessed by measuring serum ferritin) were related to the rate of change in regional bone loss over a 3-year period (482). This finding supports what we have observed during space flight (469). Other risks with iron overload are retinal degeneration and cataract risk (483). We have seen that, in rat studies of iron loading plus radiation exposure, increased oxidative damage occurs in the retina as well as systemically and in the liver (484). Furthermore, the formation of free radicals subsequent to elevation of iron stores has been linked on Earth to cardiovascular disease and cancer. Although aspects of some of the evidence supporting this thesis contradict each other (485, 486), a correlation between coronary heart disease and iron status has been described in a number of recent studies (487-489), and an association between increased incidence of myocardial infarction and increased iron stores (as measured by serum ferritin) has been observed (489, 490). In a prospective Finnish study, increased risk of all cancer types combined and colorectal cancer in particular was associated with high iron stores (491). The relationship between iron, lipids, and cancer has also been documented in the Framingham study (492). A relationship has also been indicated between excessive iron stores and ascorbic acid deficiency; when reductions in ascorbic acid occur, vitamin A and selenium tend to exacerbate iron-induced peroxidation processes (493). These data suggest that the alterations in erythropoiesis and iron metabolism that occur in microgravity could cause significant changes affecting crew health.

Better characterization of iron metabolism during space flight with respect to other systems is warranted because of the high levels of dietary iron, the increase in iron stores early during flight, and the potential for iron to act as an oxidizing agent during space flight, complicated by increased radiation levels. It is known that bacterial virulence increases upon exposure to microgravity (494), and ground studies also show that increased iron status can increase risk for infection (495). Investigating the increase in iron status during flight with respect to changes in immune function will be an important next step in understanding the implications of elevated iron status during space flight. Furthermore, iron absorption has yet to be determined during flight.

Copper

Copper is an essential cofactor for enzymes involved in energy production, metabolism of oxygen and iron, maturation of the extracellular matrix and neuropeptides, and neuroendocrine signaling (496). Deficiencies in copper have implications for bone health, the nervous system, immune function, the cardiovascular system, and lipid metabolism (496). The involvement of copper in bone health is specifically related to lysyl oxidase function and collagen synthesis (271, 496).

Copper is not usually stored in tissues, but liver, brain, and kidney typically contain the largest amounts per unit tissue mass (496). Total body copper is about 50 to 120 mg (0.79-1.9 mmol) (497). Copper transport and regulation involve the blood protein ceruloplasmin.

Frank copper deficiency is rare in human populations consuming a normal diet; however, copper deficiencies have been noted in infants fed milk formulas, infants recovering from malnutrition and fed cow's milk, and patients receiving total parenteral

nutrition for a prolonged period (498). Six patients fed (through the gastrointestinal tract) a diet containing 15 µg copper/100 kcal for 12 to 66 months (499) developed a copper deficiency.

When copper deficiency occurs it leads to normocytic, hypochromic anemia; decreased production of leukocytes and neutrophils; and defects in connective tissue (specifically in collagen synthesis) that can lead to vascular and skeletal problems and central nervous system dysfunction, or even death (498). Heartbeat irregularities have also been reported in cases of copper deficiency (500). Deficiency symptoms, including macrocytic anemia, bone abnormalities, and decreased neutrophil production, have been reported in subjects with serum copper concentrations ranging from 0.9 to 7.2 µmol/L (499). Toxic concentrations of copper lead to oxidative damage, gastrointestinal distress, liver damage, or even death (496).

Serum copper and ceruloplasmin of ISS crews have been determined as part of the medical requirement to assess nutritional status in long-duration crewmembers, with no significant changes observed after flight (6). In-flight determinations of copper status have not yet been reported.

One Russian report on the effect of space flight on copper content of bones (431) documented "non-uniform changes" in copper content of bone from different regions after flight relative to nonflight controls. Copper content of the femoral epiphysis was 81% to 159% greater, while the amounts of copper in the vertebral body and sternum were 36% and 58% less, respectively. (This study reported on the autopsy results after the tragic end of the 24-day Salyut-1 mission, relative to controls.)

During a 17-week bed rest study, copper balance was unchanged, but after re-ambulation it increased (435). During and after 3 weeks of bed rest, serum copper and ceruloplasmin were unchanged (134). After 90 days of bed rest, serum copper was slightly elevated, but the change was statistically significant (283). In 60- and 90-day bed rest studies, ceruloplasmin was unchanged (283).

Changes in copper status could contribute to the effects of space flight on bone, red blood cells, and iron status. The changes in bone during space flight, described in this volume, could be exacerbated by copper deficiency and impaired collagen synthesis. Anemia of space flight is manifested as a reduction in circulating red blood cell mass with elevations in serum ferritin and iron concentrations (35, 461). Since copper is required for iron mobilization and absorption, alterations in copper status may affect iron and red blood cell changes during flight.

Appropriate amounts of certain nutrients, copper in particular (501), are vital for maintaining normal immune function. The immune system seems to be altered during space flight (77, 502-505), and this may have direct or indirect (when alterations are induced by stress or radiation) implications for nutrition and nutritional status as possible causes or effects (503, 506).

No information about copper absorption and metabolism during space flight is available, but, given the available ground data, obtaining such information is not a high priority at this point. Ensuring adequate copper content of the diet and verifying that the flight data on copper status follow ground trends are important monitoring steps.
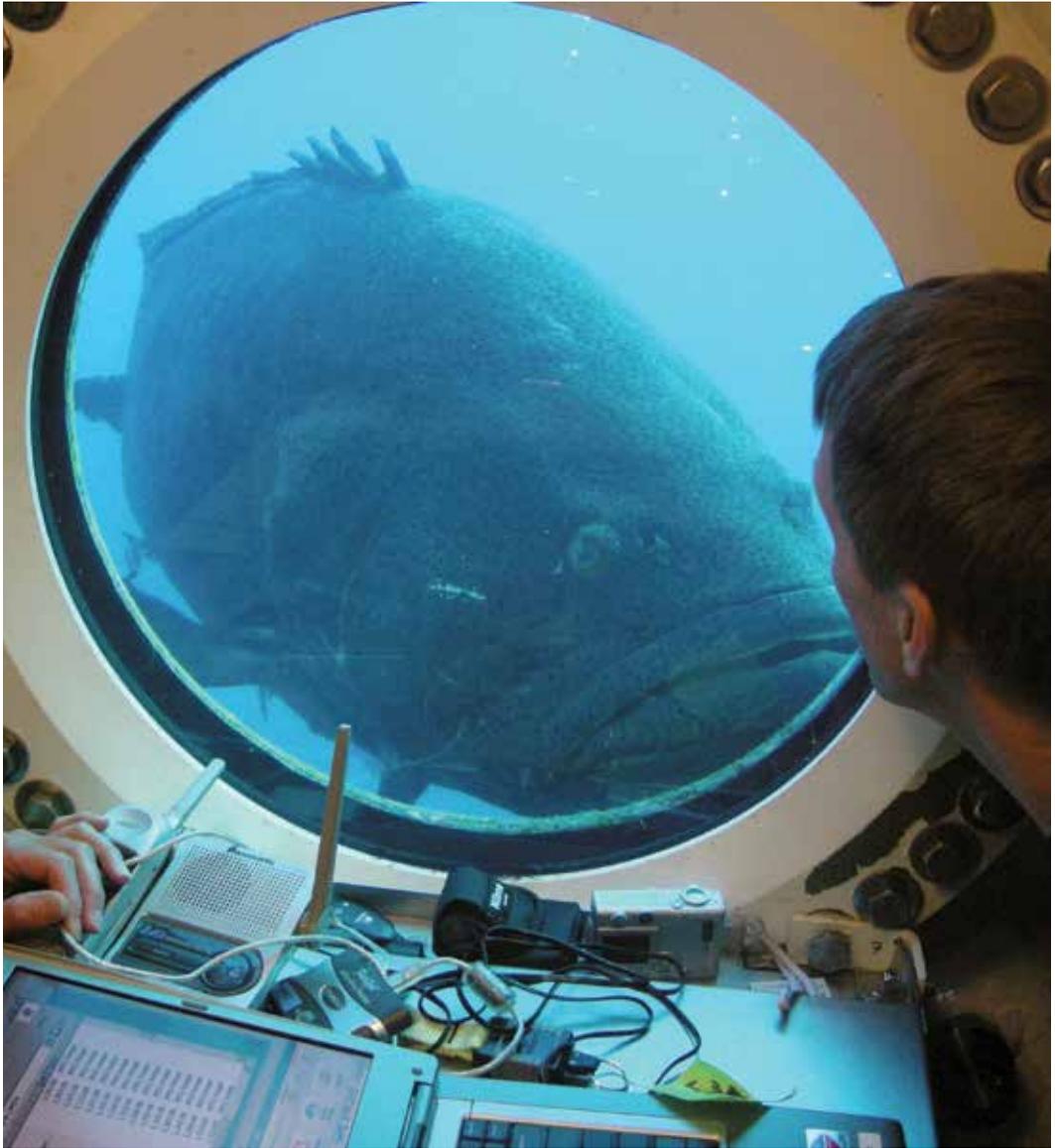
Josef F. Schmid, NASA flight surgeon, comes face-to-face with a grouper through the observation window of his undersea habitat during the 12th NASA Extreme Environment Mission Operations (NEEMO) mission aboard the National Oceanic and Atmospheric Administration's Aquarius Underwater Laboratory, which is operated by the University of North Carolina at Wilmington and located off the coast of Key Largo, Florida.

6 Cardiovascular Health

Cardiovascular issues are a key concern for space travelers (507-513), but the role of nutrition in cardiovascular adaptation has not (yet) been well characterized. In a few areas there is some degree of evidence (described below), but many more have yet to be examined. It is worth noting that multiple studies are being planned or are underway on ISS and in bed rest models that may help shed light on this area in the near future.

Energy

As discussed in section 2, "Energy and Fuel Metabolism," striking data show that cardiovascular deconditioning and loss of plasma volume are negatively correlated with energy consumption during bed rest (63) and after space flight (Figure 33). That is, insufficient energy intake is associated with greater plasma volume loss and cardiovascular deconditioning. The space flight data came from the work of Dr William Carpentier, who evaluated crewmember medical records from the Mercury, Gemini, and Apollo programs. These data have yet to be published in the scientific literature, but a book in which they are compiled is in final development.

Dr Carpentier's data from astronauts in the early US space programs have been integrated and modeled to predict postflight heart rate response to lower body negative pressure (LBNP), standing, and tilt from factors including flight duration, plasma volume loss or energy intake, and preflight resting heart rate. The plots in Figure 33 and Figure 34 are based on Gemini and Apollo mission data. Combined with the data presented in Figure 35, these data clearly link energy intake and plasma volume loss with cardiovascular health during and after space flight.
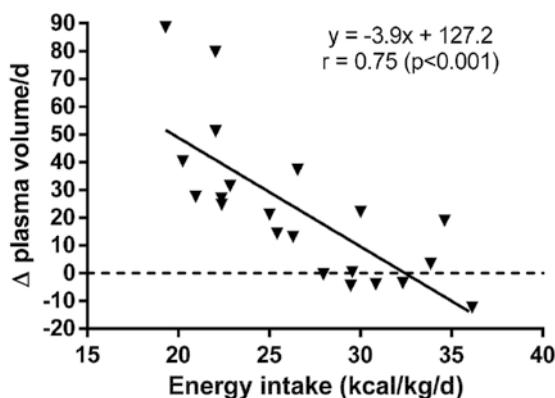
Figure 33. Relationship between energy intake (kcal/kg body mass/d) and plasma volume loss (mL/d) during Apollo missions. N = 21. Data are courtesy of William Carpentier.
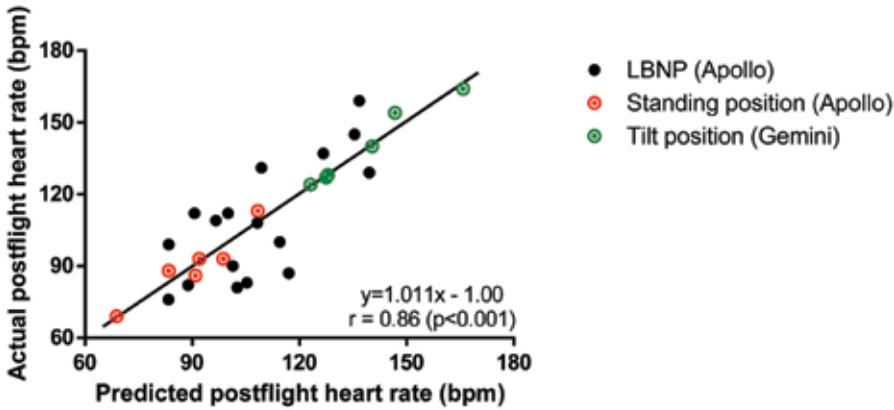
Figure 34. Postflight heart rate under 3 conditions, predicted (from factors including plasma volume loss) versus actual measured heart rate. These data were calculated from the following equations:

LBNP: 29.00 – (0.064 x flight duration) + (1.45 x resting preflight heart rate) + (4.2553 x plasma volume loss, mL/h flight)

Standing: 77.23 – (0.52 x flight duration) + (1.9098 x preflight resting heart rate) + (17.4758 x plasma volume loss, mL/h flight)

Tilt: 330 – (2.53 x preflight resting heart rate) – (7.20 x plasma volume loss, mL/h flight) – (0.078 x flight duration, h)

Heart rates for LBNP and tilt were those recorded at LBNP = -50 mm Hg and at 70° tilt. For the stand test, crewmembers stood relaxed against a wall with their heels 6 inches away from the wall. Data are courtesy of William Carpentier.
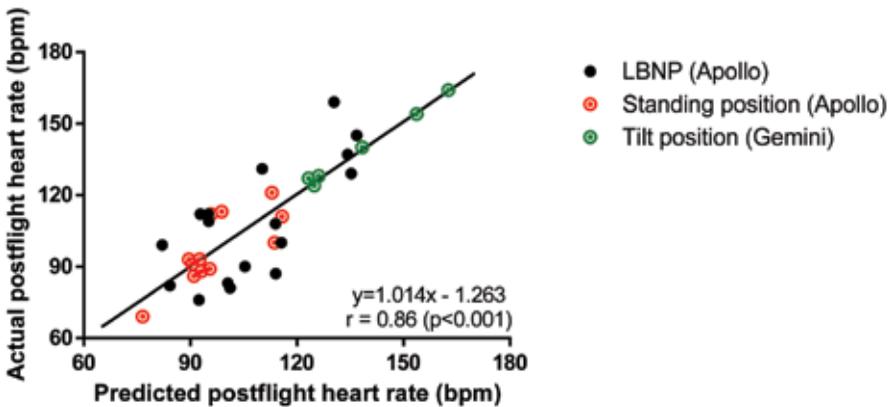
Figure 35. Postflight heart rate, predicted (from factors including energy intake) versus actual measured heart rate. These data were calculated from the following equations:

LBNP: 63.02 + (1.18 x preflight resting heart rate) + (0.48 x energy intake, kcal/d/kg) – (0.17 x flight duration)

Standing: 115.87 + (1.12 x preflight resting heart rate) – (2.17 x energy intake, kcal/d/kg) – (0.17 x flight duration)

Tilt: 239.48 – (0.75 x preflight resting heart rate) – (1.86 x energy intake, kcal/d/kg) – (0.052 x flight duration)

Similar findings relating energy intake and cardiovascular deficits were obtained from bed rest studies led by Dr Martina Heer to evaluate the effects of hypocaloric diets on many physiological systems (61, 183). The cardiovascular data showed that caloric restriction during bed rest led to decrements in cardiovascular physiology (specifically, performance on a stand test), exceeding the decrements that occurred during bed rest when subjects received adequate calories (unpublished results).

One piece of the space flight puzzle that is still missing is the effect of flight duration. That is, the data presented in Figure 33, Figure 34, and Figure 35 were generated from short-term flights, and this relationship may change on longer ISS missions. Given the inference from Figure 33 that energy intake should be greater than 33 kcal/kg body mass to avoid plasma volume loss, we evaluated ISS intake data (Figure 36) and found that few crewmembers are meeting this threshold.
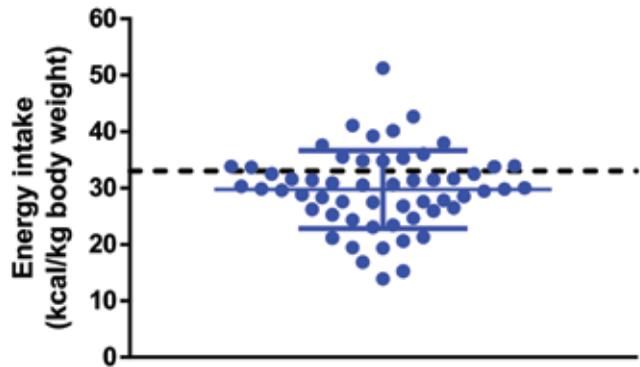
Figure 36. Energy intake during space flight on ISS missions (N=56). Each point represents an individual crewmember, and is their reported average daily energy intake over the course of the mission. The dashed line represents 33 kcal/kg body mass, the energy intake needed to avoid plasma volume loss. Solid lines represent the mean ± 1SD.

Magnesium

As mentioned in other sections, magnesium has benefits for metabolism of bone and calcium and for reduction of renal stone risk. In addition, magnesium has been shown to have effects on the cardiovascular system (514-516). Consistently decreased magnesium excretion after flight is a concern for many reasons (see section 4, "Bone," for details), and warrants further investigation with respect to cardiovascular deconditioning during and after flight.

Ongoing and Future Research

Oxidative Stress

The role of oxidative stress and antioxidants in cardiovascular adaptation to space flight is currently being studied on ISS. Although astronauts are exposed to several oxidative stressors during flight (eg, radiation, EVA, iron stores, exercise), abundant evidence exists of additional oxidative stress during space flight (reviewed later), and a wealth of literature reports effects of oxidative stress and damage on the cardiovascular system on Earth, these three areas have never been evaluated. A study initiated on ISS in 2013 aims to accomplish this goal, evaluating oxidative damage during flight, as well as evaluating long-term effects after flight.

The findings from these studies may have significant implications for future missions. As described later in this book, oxidative stress is a multifaceted issue that affects many systems, and given the radiation concerns of exploration-class missions, it will draw greater attention in the future.

Omega-3 Fatty Acids

Omega-3 fatty acids have a clear beneficial impact on cardiovascular health on Earth, but such effects have not been evaluated during space flight. Nonetheless, the initial efforts being made to increase fish and omega-3 fatty acid intake in astronauts for the benefit of other systems (bone, muscle) will likely have positive effects here as well.

Healthier Diets

While individual nutrients are easier to study in a controlled, experimental fashion, the effect of overall dietary quality is one topic continuing to gain ground, particularly as studies of individual supplements fail to produce the "magic" supplement. Overall dietary quality, including fruit and vegetable intake, fish (omega-3 and vitamin D) intake, and intake of foods rich in phytochemicals and lower in sodium, has broad health effects (517-522). Many of these effects clearly have an impact on the cardiovascular system, along with benefits for bone, muscle, kidney, and other systems.

In 2014, NASA embarked on an "Integrated Nutrition" study, aimed at providing healthier food options for astronauts on ISS missions. As of this writing, it is hoped that the first flight of this experiment will be in 2015. Bone is a primary end point, but clearly this study will have implications for many other physiological systems (eg, cardiovascular, muscle, immune) and other risks of space flight (eg, radiation and oxidative stress), not to mention potential benefits for crew performance and morale. The outcome of this study will provide a solid backdrop for the definition of food system and nutrient requirements for exploration missions, and may also have significant implications for understanding the role of nutrition in disease prevention here on Earth.

NASA astronaut Karen Nyberg, Expedition 36 flight engineer, eats a meal at the galley in the Unity node of the International Space Station.

7 Sodium, Potassium, Fluid, and Renal Stone Risk

Sodium and Chloride

Sodium is the major cation of extracellular fluid (523). Together with chloride, sodium is used by the body to maintain normal water distribution, osmotic pressure, and anion-cation balance in the extracellular fluid compartment (524). Electrolyte concentrations in the body are essential for proper cardiovascular function and are under renal and hormonal control (525). Increases in blood sodium levels can be caused by diabetes, renal polyuria, diarrhea, insufficient water intake, excessive sweating, or increased dietary sodium intake. Sodium levels decrease with edema, excessive water intake, vomiting, diarrhea, diuretic therapy, renal tubular damage, hyperaldosteronism, or lower dietary intake.

For the normal adult, total body sodium averages about 60 mmol/kg body weight. Forty to 45 percent of total sodium resides in bone, with the balance found in extracellular and intracellular fluid. These sodium stores are classified as either exchangeable (42 mmol/kg body weight) or nonexchangeable, the exchangeable stores being composed of all cellular sodium and less than half of bone sodium (526). Exchangeable sodium becomes available by diffusion when plasma sodium levels become low, and in states of edema, the exchangeable sodium stores absorb sodium.

Animal studies show that symptoms of sodium deficiency occur after 3 to 4 weeks of dietary sodium restriction (527). During acute starvation, urinary sodium excretion decreases to less than 0.2 g within 10 days (528), and can be affected by the amount of sweat (529). Plasma sodium levels are maintained fairly well during acute starvation: an initial decrease is followed by a return toward normal values (530). Maintenance of blood sodium is also observed during semi-starvation. During the Minnesota Experiment, plasma sodium levels in samples taken after the 6-month semi-starvation period were 0.6 ± 7.3% higher than baseline levels (N = 4) (529). Six days of undernutrition resulted in large negative balances of sodium chloride (–12.8 ± 3.6 g/d), likely related to changes in water balance (529).

Pre-, in-, and postflight plasma sodium and chloride data are available from Apollo, Skylab, and Shuttle flights, and have been reviewed extensively (2, 21, 531-534). In-flight daily sodium intakes during Skylab and Shuttle missions averaged 4 to 5 g, and were similar to the astronauts' preflight intakes (32). The current food system is high in dietary sodium, and typical intakes on ISS have been in excess of 4.5 g, even with suboptimal food intake (6). Intakes as high as 7 to 10 g of sodium per day have been observed. Sodium homeostasis and blood sodium levels are maintained during real and simulated space flight (535), but the high sodium content of the current space food system makes it important to monitor and restrict dietary sodium intake of astronauts to maintain their bone and renal health.

European studies with Mir crewmembers documented positive sodium balance during space flight, in a non-osmotic fashion (that is, without a concomitant increase in fluid compartments) (21, 30, 533, 534, 536, 537). These data were confirmed in a series of ground-based studies, documenting an increase in messenger RNA expression of some

of the enzymes required for glycosaminoglycan syntheses in the skin, the displacement of sodium by hydrogen in the glycosaminoglycans, and a subsequent acidosis (537-543). These findings of increased sodium-proteoglycan binding had already been observed in animal studies by Ivanova in the 1970s (544). The skin here functions as a reservoir that stores sodium in case of overconsumption and releases sodium when it is insufficiently supplied. In recent studies carried out by the group of Titze et al (538, 539, 541) in animals and summarized in a mini-review (541), they demonstrated the involvement of the lymph capillary system in the clearance of sodium and chloride from the skin. Increasing density of lymph capillaries in the skin seemed to play one of the key roles in this clearance, and when hyperplasia of the cutaneous lymph capillary system was inhibited, skin sodium and chloride retention was augmented and led to increased blood pressure.

Sodium is also stored in bone; however, sodium stored in bone does not seem to be exchangeable and therefore does not take part in day-to-day sodium regulation. However, on Earth, excessive sodium intake has been associated with increased bone turnover (545-547). Dietary sodium is known to affect calcium homeostasis (548-553). A predictable relationship exists between urinary sodium and calcium; that is, for each 100 mmol of sodium excreted in urine, 1 mmol of calcium is excreted (554). This phenomenon is expressed at high levels of dietary sodium. More than 90% of dietary sodium is absorbed, even when intake is high (555). Sodium is excreted mostly in the urine, but about two-thirds of the sodium filtered by the kidney is reabsorbed by mechanisms thought to involve solvent drag and electrochemical gradients. The sodium-dependent calcium transport system uses the energy stored in the electrochemical gradient of sodium to drive calcium into the lumen of the proximal renal tubule, and ultimately the presence of calcium in this location leads to increased calcium loss secondary to increased sodium excretion. In the distal tubule, calcium is preferentially reabsorbed, an event stimulated by parathyroid hormone (PTH) and cyclic adenosine monophosphate (cyclic AMP) (556). Cyclic AMP also influences reabsorption of sodium (557).

A small amount of sodium is excreted in feces. When 550 mmol sodium was ingested each day for 7 d, the average fecal excretion was 1.8 ± 0.4% of the total dose, and when smaller amounts of sodium were ingested (50 mmol/d), an average of 6.0 ± 1.0% was excreted in the feces (2).

Salt loading alone increases intestinal calcium absorption. In hypoparathyroid patients, dietary salt increased intestinal calcium absorption in one study by Meyer (558) but not in another study by Breslau (559). In Breslau's study, calcium absorption correlated with serum 1,25-dihydroxyvitamin D. Thus, conclusions about the role of PTH in the increase in intestinal calcium absorption after a sodium load are speculative.

Studies in premenopausal women suggest that increased intestinal calcium absorption, rather than increased bone resorption, compensates for sodium-induced hypercalciuria in subjects whose adaptive processes related to bone metabolism are intact (560, 561). Ginty et al (560) examined the effects of 7 days of high or low dietary sodium on bone markers in young women. Although urinary calcium was increased with high (180 mmol/d) sodium intakes, the effect of high sodium on markers of bone resorption was not different from the effect of low (80 mmol/d) sodium intakes. Lietz et al (561) also found no effect of intakes of 170 mmol/d or 60 mmol/d of sodium for 8 days on bone resorption markers in postmenopausal women. However, Evans et al (547) reported that postmenopausal women ingesting 300 mmol sodium per day for 7 days had greater excretion of bone resorption markers than those ingesting 50 mmol sodium per day.

These differences were not observed in a premenopausal group (547). These results suggest that bone resorption is increased in situations where the adaptive responses of bone are limited or altered, as they are after menopause. It might also suggest that at levels above 200 mmol sodium intake per day, the regulatory processes are different.

Data from human and animal studies suggest that high dietary sodium chloride leads to bone loss due to increased bone resorption (562-568), and even that restriction of dietary sodium will reduce bone resorption (569). In a review of the interactions between dietary salt, calcium, and bone, Massey and Whiting (567) suggested that habitual excessive salt intake contributes to bone loss. Other reviewers have come to the conclusion that increased dietary sodium chloride intake negatively affects acid-base balance, with subsequent loss of calcium (570, 571).

Massey and Whiting (567) found that the effect of excessive salt intake on bone loss is modulated in specific subpopulations. For example, people who tend to form renal calcium stones are more responsive to changes in dietary salt than are non-stone formers. Although sodium intakes of stone formers are typically similar to those of controls (572, 573), the detrimental effects of high sodium intakes on renal stone risk have been well documented (562, 568, 571). Increasing sodium intake from 50 mmol/d to 300 mmol/d increased renal stone risk by elevating urinary saturation of calcium phosphate and monosodium urate, and reducing inhibition of calcium oxalate crystallization (574).

Work by Goulding (545, 546) and Matkovic (575) has generated interest in the effect of dietary sodium on bone mass. High levels of dietary sodium are not only major predictors of urinary calcium and hydroxyproline excretion, but are also associated with greater loss of bone with age, unless dietary calcium is supplemented (576). Work by Dr Heer's group has also documented the resorptive response to high dietary sodium, and the role of acid-base balance in this process (577).

Dietary sodium also seems to exacerbate the calciuric responses to musculoskeletal unloading in weightlessness (Figure 37). Bed rest subjects consuming a low-sodium diet (100 mmol/d) had no change in urinary calcium, while those on a high-sodium diet (190 mmol/d) had hypercalciuria (578). A more recent bed rest study by Heer et al documented that the high-sodium-induced increase in bone resorption exceeded the bed rest-induced increase, through a mechanism mediated by acid-base balance (577, 579, 580). One mechanism could be that metabolic acidosis causes an increase in urinary corticosterone (581). Increased sodium intake and consequent low-grade metabolic acidosis caused an increase in bioactive glucocorticoids (540, 582). In turn, the increase in glucocorticoid concentration caused rapid bone loss (583-586). Applying an alkaline salt together with high sodium intake (582) reduced bioactive glucocorticoid excretion, which result supports the idea that acid-base balance plays a role in the effects of high sodium intake. A symposium was held recently in Germany (its proceedings were published in 2008) regarding the impact of acid-base balance on health issues (282), including the role of sodium in bone loss (587-589).

High sodium intakes during space flight can exacerbate bone loss and lead to increased risk of renal stone formation. In and of itself, excess sodium can lead to hypernatremia, hypertension, and even death. Although it has not been a concern to date, too little dietary sodium or a deficiency of this electrolyte during flight could lead to hyponatremia, hypotension, and even death.
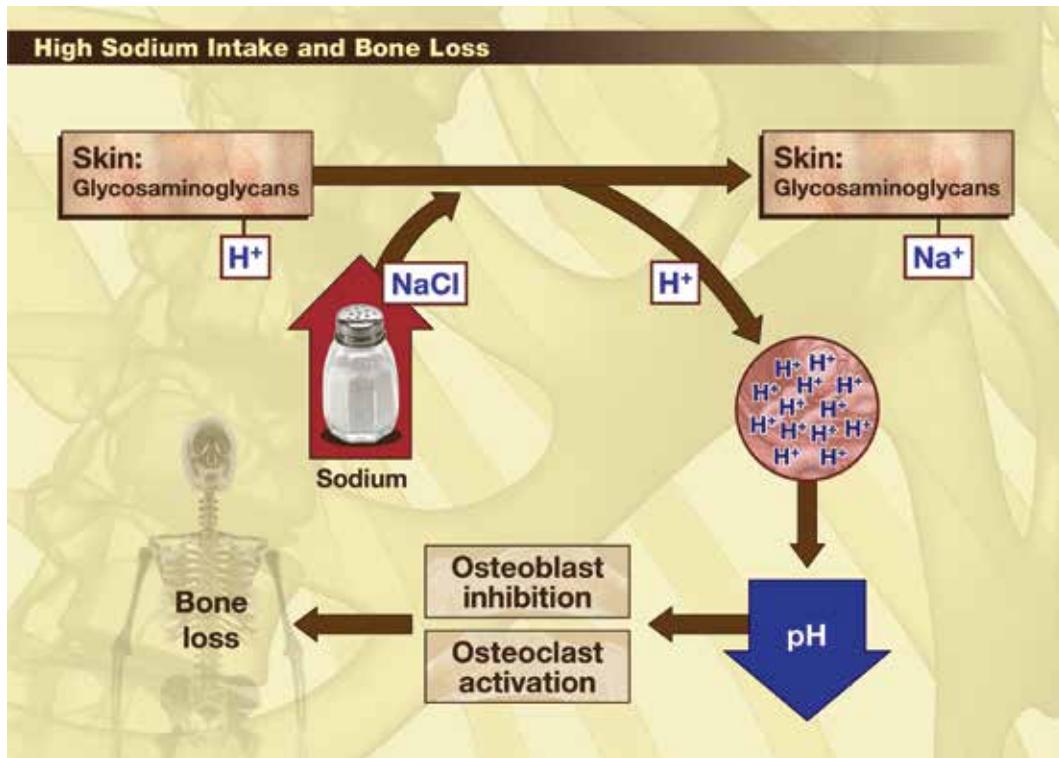
Figure 37. Proposed mechanism of the effects of high dietary sodium on bone loss.

Further research is required to investigate potential effects of high sodium intake during space flight, as the space food system currently has very high sodium levels. The impact of high sodium intake on bone, calcium, and pH is not well understood, and adjustments in sodium intake may serve as a viable countermeasure to bone loss. Furthermore, the role of a high-sodium diet in potassium homeostasis is not well understood. This may prove to be an area where nutrition and cardiovascular effects of space flight may interact, and study of the interaction may produce a dietary countermeasure.

Potassium

As the most plentiful intracellular cation, potassium has a significant role in several physiological processes (525). It is crucial to regulation of acid-base balance, energy metabolism, blood pressure, membrane transport, and distribution of fluid within the body. It is also involved in the transmission of nerve impulses and cardiac function (590). Potassium metabolism that is disordered because of excessive or deficient circulating levels has negative consequences for cardiac, muscle, and neurological function.

Deficiency of potassium leads to hypokalemia, muscle weakness, constipation, and fatigue, or even death. No evidence of adverse effects is associated with toxicity of potassium from naturally occurring sources. However, supplemental intake may cause hyperkalemia (and associated weakness, cardiac arrest, and paralysis), metabolic acidosis (282), decreased neuromuscular function, or even death.

Serum and urinary levels of potassium were both decreased after space flight in Apollo crewmembers (591), and evidence exists that a similar decrease occurred in Skylab crewmembers (29).

Loss of both total body potassium and exchangeable potassium was observed in Apollo crewmembers (591). Increased levels of urinary potassium during space flight may be related to muscle disuse atrophy and inadequate intake (535). In the initial days of bed rest, excess dietary sodium was shown to be potassium-depleting (Heer et al, unpublished observations). Loss of lean body mass, along with high sodium intake, may also result in potassium depletion.

The relationship between bone health and the protein:potassium ratio in the diet needs to be further investigated, along with the role of potassium in cardiovascular health during flight.

Fluid

Adequate fluid intake is necessary to maintain the body's normal hemodynamic state and normal fluid osmolality, which are important for cardiovascular health and for maintenance of fluid and electrolyte homeostasis. Water is a structural component of the body and the solvent for transportation of nutrients and waste. Fluid and electrolytes may be lost from the body by a variety of routes and for a variety of reasons. They are excreted in sweat, urine, and feces, and in abnormal situations excessive amounts can be lost by these routes and others. Significant losses may occur through the gastrointestinal tract as a result of diarrhea, vomiting, or gastric drainage. Loss through the skin increases with fever, increased metabolism, sweating, and burns (523).

Total body water makes up about 50% to 70% of body mass (592). Fluid requirements increase with metabolic rate and heat stress. Death from dehydration can occur within weeks of depriving the body of all water (593).

Fluid and electrolyte homeostasis is significantly altered during space flight, and this has been extensively reviewed (19, 30, 531, 594-600). The hypothesis originally proposed to explain this was that upon entering weightlessness, the human body would experience a headward shift of fluids, with subsequent diuresis and dehydration. A series of flight experiments was conducted to assess fluid and electrolyte homeostasis during space flight; the most comprehensive of these took place on the 2 Spacelab Life Sciences missions in the early 1990s. Despite much research, the hypothesis of diuresis and subsequent dehydration secondary to the headward fluid shifts has never been confirmed during actual space flight (19, 531, 533, 534, 599, 601).

Within hours of the onset of weightlessness (the earliest available data point), a reduction in both plasma volume and extracellular fluid volume had occurred, accompanied by the "puffy" faces typically observed early in flight (19, 602). Initially, the decrement in plasma volume (~17%) was larger than the decrement in extracellular fluid volume (~10%), suggesting that interstitial fluid volume (the other four-fifths of extracellular fluid) is conserved proportionally more than plasma volume (19). The idea that interstitial fluid volume is conserved is supported by rapid decreases in total circulating protein, specifically albumin (19), indicating that protein, and associated oncotic pressure, shifted from the intravascular to the extravascular space. This would have facilitated the initial changes in plasma volume (19).

Following the initial adaptation, extracellular fluid volume further decreased between the first days of flight and 8 to 12 days after launch, from the initial ~10% below preflight levels to ~15% below preflight levels (19). Plasma volume was partially restored during this period, from the initial ~17% below preflight levels to ~11% below preflight levels (19), and it has been found to remain 10% to 15% below preflight levels even for extended-duration flights (603).

Leach et al (19) and Norsk et al (534) have hypothesized that the shift of protein and fluid to the extravascular space represents an adaptation to weightlessness, and that after several days, some of the extravascular albumin has been metabolized, with a loss of oncotic force and a resulting decreased extracellular fluid volume and increased plasma volume. This loss of extracellular protein (intra- and extravascular) and the associated decreased oncotic potential probably play a role in postflight orthostatic intolerance, which has been considered to result partly from reduced plasma volume at landing (604). Furthermore, the loss of protein may in part explain why fluid loading alone does not restore circulatory volume (605, 606), as no additional solute load exists to maintain the fluid volume. Another potential (or perhaps partial) explanation for the failure of fluid loading is that because astronauts' diets are high in sodium, additional salt cannot help increase plasma volume or extracellular fluid volume. This explanation has been documented in bed rest (537).

The effect of space flight on total body water has been evaluated to assess hydration. Shuttle and Skylab astronauts had decreases of about 1% in total body water during flight (19, 607, 608), and the percentage of body mass represented by water did not change. Thus, the often-proposed weightlessness-induced dehydration does not exist. This has also been shown by European investigators on Shuttle and Mir missions (534, 536, 599, 601, 609).

Diuresis is also typically not observed during flight (59, 531, 534, 536, 594, 601, 610-612), for a number of possible reasons. Operational constraints have made it difficult to document urine volume accurately on the first day of space flight. However, on the Spacelab Life Sciences missions, urine volume on the first 3 days of flight was significantly less than preflight volume, and urine volume tended to be less than preflight volume throughout the flight (19). Urine volumes on a week-long flight to Mir were also less than preflight volumes (611). During the first week of the 59- and 84-day Skylab flights (29), urine volume was less than it was before flight, and for the remainder of the missions it was unchanged from preflight levels. Decreased fluid intake likely accounts for the decreased urine volume, which was accompanied by little or no change in total body water. Adequate urine volume during flight is important for reducing the risk of renal stone formation (298, 299, 613, 614).

As mentioned above, the percentage of body mass represented by total body water is relatively unchanged during flight (19). However, on a volume basis, the change in extracellular fluid volume was found to be greater than the change (or lack of change) in total body water (19). Thus, by difference, intracellular fluid volume increased during space flight. This had been previously hypothesized from ground-based studies (615) and observed in postflight studies of Apollo crewmembers (59). The mechanism for a space flight-induced increase in intracellular fluid volume is unknown. One possible explanation is that a shift in fuel utilization results in increased glycogen storage, a condition known to increase cellular water content.

Diuresis has been documented to occur in bed rest studies (616-618). Urinary albumin, a marker of kidney function, has been shown to be reduced in both space flight (relative to before flight) and bed rest (relative to the ambulatory state) (157-159). However, space flight, but not bed rest, results in reduced urine flow rates (601). Taken together, these data suggest that differences in fluid metabolism exist between analog studies and actual space flight (533, 534, 599, 601, 609, 612, 618). Such differences do not seem to be a simple effect of abnormal renal function, and thus require further investigation (619).

Although no space flight-induced dehydration occurs, care must be taken to ensure adequate fluid intake and hydration status. Inadequate fluid intake increases the risk

of dehydration and renal stone formation. Fluid intake during flight is typically less than preflight intake, and often below the recommended quantity. In closed flight vehicles, water is often a limiting resource, but rationing of water should be avoided.

Deficiency of fluid leads to dehydration and ultimately death. Likewise, an excess of fluid intake leads to water intoxication and ultimately death. Obviously, the risk of this occurring during space flight, where water is a limited commodity, is extremely low.

Decreased fluid intake during space flight may be a consequence of reduced thirst during flight (86), but the reason for reduced thirst is unknown.

Studies described above have documented that total body water is unchanged during flight, but apparently a shift of fluid from the extracellular to the intracellular compartment occurs. The effect of this shift on cell size and cell function (such as the effect of a change in the density of receptors on cell membranes) has not been evaluated. A change in cell size and function might be responsible for some of the microgravity-induced changes noted in other systems (such as the endocrine, cardiovascular, and immune systems).

Renal Stone Risk

A renal stone risk profile is determined from measured urinary oxalate, uric acid, citrate, calcium, sodium, magnesium, sulfate, potassium, pH, phosphorus, and total volume, and calculated supersaturation of calcium oxalate, brushite (calcium phosphate), sodium urate, uric acid, and struvite (magnesium ammonium phosphate). Generally, renal stone risk is elevated during space flight (299). As with any space flight effect, some crewmembers are more affected than others. Figure 38 is an important illustration of this. Some crewmembers had very high elevations in brushite or calcium oxalate supersaturation during space flight, whereas others had very low levels of supersaturation. During space flight, environmental and dietary factors all greatly affect renal stone risk. Crewmembers who have an increased risk before space flight and then are exposed to microgravity with concomitant bone loss, hypercalciuria, increased urinary sodium, and decreased urinary output may have a further increased risk of renal stone formation during space flight.
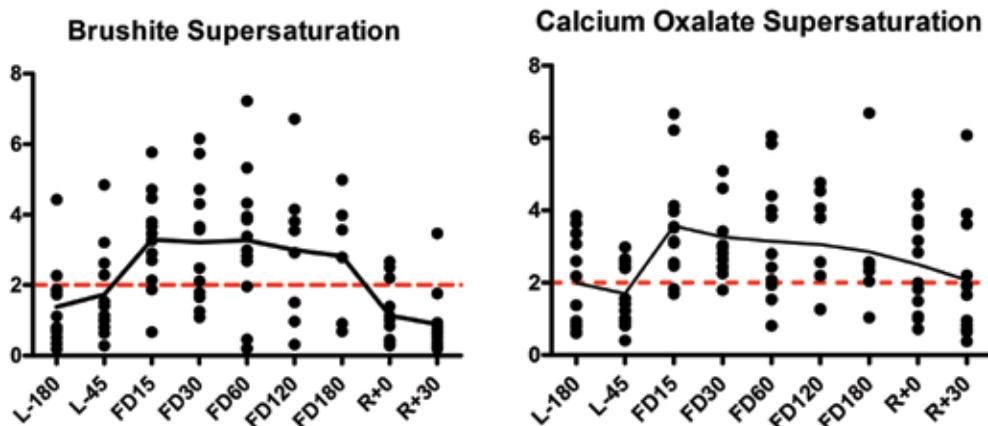
Figure 38. Estimated supersaturation risk from the Renal Stone Risk Profile during space flight on ISS. Each symbol represents a 24-h urine pool, and the solid black line represents the group mean. The red dashed line is the point above which the risk is greater than in the non-stone forming population.

As of 2013, 25 renal stone events have been reported among 19 crewmembers with the majority of these stone events occurring post flight (Robert Pietrzyk, personal communication). To minimize the risk of stone formation, potassium citrate (KCit) has been successfully tested during ISS missions (433), and has been "transitioned to operations." This means that KCit is part of the flight surgeon's toolbox for mitigating the renal stone risk of crewmembers and is available on the ISS for use at flight surgeon discretion if clinically indicated. The use of KCit was shown to increase the urinary pH, increasing the solubility of uric acid and thereby decreasing the risk of uric acid stone formation. The dosage of KCit must be carefully prescribed so as not to increase the risk of brushite stone development due to elevated urinary pH. However, given that fluid intake for maintenance of hydration is a preferred countermeasure, and some residual concerns exist about potassium supplementation side effects, it was decided not to routinely provide KCit to crewmembers.

Magnesium and citrate are both considered protective when it comes to calcium-containing renal stone risk. However, they do not minimize risk for all forms of kidney stones; specifically, these urinary stone inhibitors do not reduce the risk of sodium urate kidney stones. Thus, taking KCit or KMgCit should not be perceived as a panacea for stone risk. In fact, in our data, sodium urate supersaturation is significantly correlated with urinary citrate excretion (Figure 39).
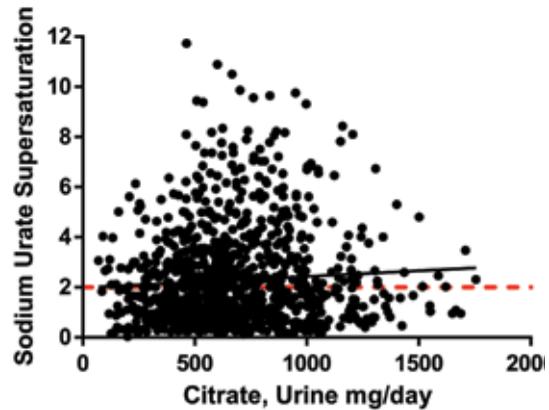
Figure 39. Relationship between urinary citrate and sodium urate supersaturation. The dashed line at 2 represents the average risk of sodium urate stone formation for the general population, with numbers above 2 being higher-than-average risk. Citrate, which typically protects against renal stone risk, is actually positively correlated with sodium urate supersaturation (Pearson $r = 0.065$; $P < 0.016$). The red dashed line is the point above which the risk is greater than in the non-stone-forming population. Data are from 1399 twenty-four-hour urine collections.

Liquid salt and pepper dispensers floating on Space Shuttle Columbia's middeck during STS-94.

8 Space Flight Ophthalmic Changes and Nutrition

Ophthalmic health among astronauts has gotten attention in recent years because of a newly identified issue for some crewmembers. In addition to a general increase in cataract risk (620-622), some crewmembers have experienced vision-related changes after long-duration space flight. These changes include optic disc edema, globe flattening, hyperopic shifts, choroidal folds, and cotton wool spots (623). The etiology of the refractive and structural ophthalmic changes is currently not known and continues to be researched, but biochemical evidence indicates that the folate- and vitamin B_{12}-dependent 1-carbon transfer pathway may be involved. Nutrition is known to be an important factor for ophthalmic health in general. This section will review the available literature on this topic and general nutrition in ophthalmic health, along with ongoing research to understand and counteract these effects of space flight.

Ophthalmic Changes

Mader and colleagues first described 7 cases among long-duration crewmembers on ISS who had evidence of ophthalmic changes after flight, including optic disc edema, globe flattening, choroidal folds, and hyperopic shifts (623). Since then, additional cases have been identified, with the prevalence approaching 20% to 30% of crewmembers. Myasnikov and Stepanova reported evidence of postflight edema of the optic nerve discs among Russian cosmonauts and 1 case (out of 10) with signs of intracranial hypertension, although they note the measurements were made before and after (not during) flight (624). The mechanism of the ophthalmic changes is not known, but microgravity-induced fluid shifts (2, 531), elevated cabin CO_2 exposure, possible intraocular pressure or intracranial pressure changes, and local intraorbital (choridal and optic nerve sheath) changes have been suggested as possible contributing factors. It is not clear why some crewmembers on some missions may experience these issues and others on the same mission, exposed to the same environment, do not. Recent biochemical evidence that the folate- and vitamin B_{12}-dependent 1-carbon transfer pathway may be involved could help explain individual susceptibility to these ocular changes.

One-Carbon Metabolism

One-carbon metabolism refers to a pathway that requires folate, vitamin B_{12}, and vitamin B_6, and is involved in purine and pyrimidine synthesis, amino acid metabolism, and synthesis of the methylating agent S-adenosylmethionine (SAM, Figure 40). Alterations in this pathway can lead to buildup of certain intermediates, including homocysteine. Factors such as genetic polymorphisms, pharmacological agents, and dietary intake and status of folate, vitamin B_6, and vitamin B_{12} can influence plasma homocysteine concentration (625, 626).

Much of the homocysteine literature has focused on its association with coronary artery disease, stroke, and migraines (625, 627, 628), but some studies show associations

of homocysteine with ophthalmic health issues. Issues such as age-related macular degeneration result from lipid deposits under the retinal pigment epithelium (629) and decreased retinal vessel functionality (630), and some theorize that age-related macular degeneration is similar to the development of cardiovascular disease (631). Because homocysteine is a risk factor for cardiovascular disease, many have looked at relationships between homocysteine, or other metabolites and vitamins in the 1-carbon metabolism pathway, and ophthalmic health issues such as age-related macular degeneration, dry eye, glaucoma, and optic neuropathy (632-635). For instance, a meta-analysis showed that elevated plasma homocysteine was associated with an increased risk of primary open-angle glaucoma (636). Other meta-analyses have shown that increased serum homocysteine and low vitamin B_{12} status were independently associated with increased risk for age-related macular degeneration (634, 637). Daily supplementation with folate, vitamin B_6, and vitamin B_{12} is associated with a 30% to 40% decreased risk for age-related macular degeneration (638).
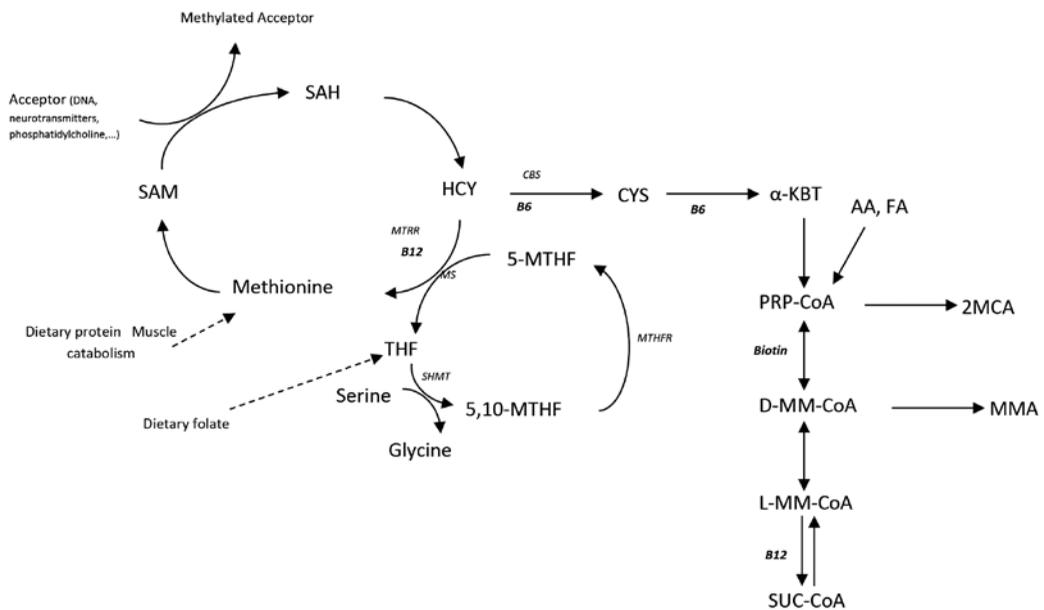
Figure 40. Overview of 1-carbon metabolism. AA, amino acids; CBS, cystathionine ß-synthase; CYS, cystathionine; FA, fatty acids; HCY, homocysteine; αKBT, α-ketobutyrate; MCA, methylcitric acid; MM-CoA, methylmalonyl coenzyme A (CoA); MMA, methylmalonic acid; MS, methionine synthase; 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTRR, 5-methyltetrahydrofolate homocysteine methyltransferase reductase; PRP-CoA, propionyl CoA; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SUC-CoA, succinyl CoA; THF, tetrahydrofolate
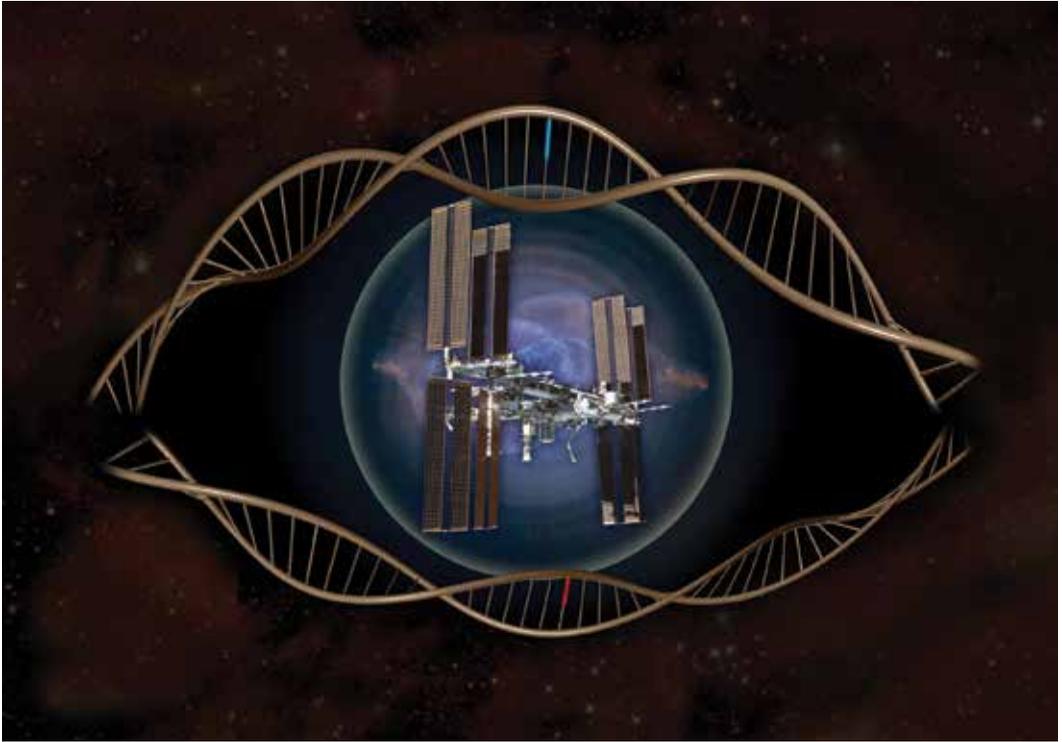
Figure 41. Artistic depiction of vision issues related to space flight, and the potential involvement of genetic influences in this problem. The depiction of a different DNA base pair (blue above and red below) in the 2 strands reflects genetic polymorphisms, or multiple forms, of the DNA. Inset imagery includes ISS and a Hubble Space Telescope image of the Cat's Eye Nebula, to reflect that these issues are relevant not only for ISS missions, but exploration-class missions as well.

Folate

Folate is the general term used to describe the vitamin folic acid and compounds that have activity similar to that of folic acid (639). Folic acid is the form of the vitamin used in vitamin supplements and fortified food products, but it is rarely found to occur naturally in food.

The reduction of folic acid and dihydrofolate by a cytosolic enzyme produces the active form of folate, tetrahydrofolate (THF). Tetrahydrofolate accepts single-carbon groups from reactions in amino acid metabolism to form active derivatives of THF (639). These derivatives function in amino acid metabolism, specifically in the reversible reactions of serine synthesis from glycine, methionine synthesis from homocysteine, and histidine metabolism. Folate is essential in cell division because the THF derivatives play important roles in purine and pyrimidine synthesis. Tetrahydrofolate derivatives play a major role in the formation of thymidylate, which is a substrate needed for DNA synthesis (639).

Deficiency of folate leads to megaloblastic anemia. Low folate intake will cause red blood cell folate concentrations to diminish within 4 months. Bone marrow cells become megaloblastic (that is, they take on a nucleated, embryonic form), and anemia occurs after 4 to 5 months of low folate intake (640). Folate deficiency in humans has been described as a 4-stage process (641, 642), including changes in serum folate (Stage 1),

changes in red blood cell folate (Stage 2), defective DNA synthesis and elevated homocysteine (Stage 3), and clinical folate deficiency (Stage 4), manifested by macroovalocytosis (many large, oval cells in the blood), elevated mean corpuscular (red blood cell) volume, and large, nucleated embryonic cells.

Early space flight data showed a reduction in red blood cell folate after long-duration missions (4, 6). Serum folate is variable among crewmembers, but generally does not change during flight (Figure 42). Interestingly, serum folate was lower during space flight in crewmembers with vision-related issues (140).
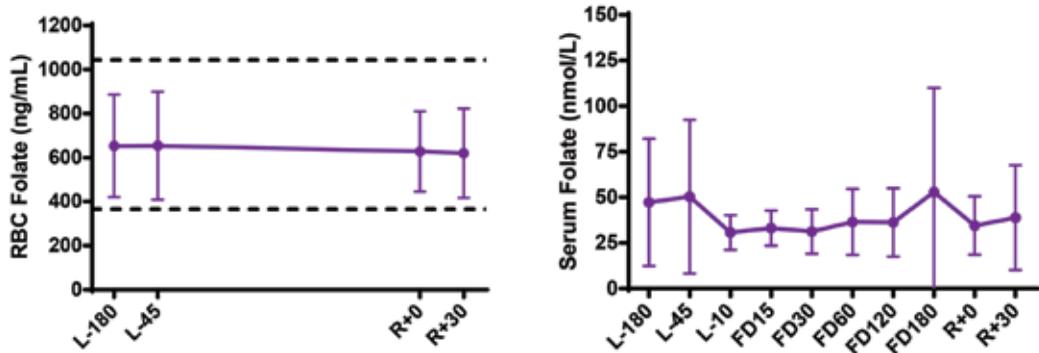
Figure 42. Red blood cell (RBC) (left) and serum folate (right) before, during, and after long-duration space flight (data are mean ± SD). Note: RBC folate data are not available during flight because of sample processing requirements.

As with many nutrients, folate deficiency on an exploration mission could be catastrophic. Animal studies have shown that low folate status increases chromosome damage resulting from radiation exposure (643-646); however, it should be noted that excessive folate supplementation provided no additional benefit (643). Similarly, cell models have shown that folic acid deficiency increases sensitivity to chromosome breakage from ionizing radiation (645). Antioxidant properties of folate have been studied, and folate was found to scavenge a diverse array of reactive oxygen species efficiently (647). Cell models also show the ability of folate to reduce iron toxicity in cases of iron overload, by oxidizing free or chelated iron (647). Evidence exists that in subjects living in saturation diving conditions with increased partial pressure of oxygen for 10 to 14 days, folate status decreases, which may be related to its antioxidant properties (481). Folate status may be even more important during exploration missions than on ISS because of known increases in iron storage during long-duration space flight (6) and exposure to ionizing radiation.

Vitamin B$_{12}$

Vitamin B$_{12}$ functions in many enzymatic reactions, and deficiencies result in anemia, as well as neurological disorders. Vitamin B$_{12}$ works as a cofactor for 3 different enzymatic reactions: (1) the conversion of homocysteine to methionine, (2) the conversion of L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA, and (3) the isomerization of L-leucine and ß-leucine. Vitamin B$_{12}$ deficiency may cause the accumulation of folate in the serum because of a reduction in B$_{12}$-dependent methyltransferase, also known as the methyl-folate trap (648). Vitamin B$_{12}$ also functions in the synthesis of choline, which can be converted to the neurotransmitter acetylcholine.

Unlike other water-soluble vitamins, vitamin B_{12} can be stored in the body for years. It is stored predominantly in the liver, but smaller amounts can be found in the muscles, kidneys, bones, heart, brain, and spleen. About 2 to 5 mg of vitamin B_{12} is stored in the body (649). The size of B_{12} stores remains relatively stable, partly because urinary and fecal excretion decrease in direct relationship to decreases in the body pools. The half-life of vitamin B_{12} in humans is 350 to 400 days (649).

No evidence of toxicity has been found with vitamin B_{12} supplementation in amounts greater than the RDA (649), and no adverse effects are reported to be caused by an excess of vitamin B_{12}. If a person went for many years without adequate intake and/or supplementation, body stores could be depleted. Other factors that could contribute to a vitamin B_{12} deficiency include a decrease in gastric acidity, the presence of atrophic gastritis, and uncontrolled growth of bacteria accompanied by malabsorption of food-bound B_{12} (650). Deficiency of vitamin B_{12} leads to pernicious anemia and demyelination of the central nervous system, and can lead to death (651).

Methylmalonic acid is generally unchanged during space flight, suggesting that vitamin B_{12} deficiency is not a significant issue during flight. However, blood concentrations of methylmalonic acid were shown to be higher in crewmembers who experienced vision-related issues after flight than in those who did not have such issues (140), and this difference was evident before, during, and after space flight. Several studies support the notion that perturbations in the vitamin B_{12} metabolic pathway can cause ophthalmic health issues such as optic neuropathy and age-related macular degeneration (632-634).

Biotin

Biotin is a required cofactor for pyruvate carboxylase, acetyl-CoA carboxylase isoforms 1 and 2, propionyl-CoA carboxylase, and ß-methylcrotonyl-CoA carboxylase (652). The 5 biotin-dependent enzymes are involved in carbohydrate, fatty acid, and amino acid metabolism (652), and the primary role of biotin is to transfer CO_2 units from one compound to another. Biotin exists in a free state or bound to proteins. About 81% of biotin in the human body is free biotin in serum, and 10% is free in tissues (653).

Despite the observation that frank signs of deficiency are rare, there is growing appreciation of genetic, physiologic, and pharmacologic conditions that marginally impair biotin status (654-656). This suggests that the lack of physiologic manifestations of biotin deficiency may not be a reliable measure to gauge biotin status. Marginal changes in biotin status have been shown to affect a range of metabolic factors, from carboxylase activity to the expression of non-biotin-dependent enzymes such as glucokinase, ornithine transcarbamylase, and phosphoenolpyruvate carboxykinase (657-659).

Biotin status has never been measured during or after long-duration space flight, but it is unlikely there are any frank biotin deficiencies, which would present as neurological and dermatological manifestations.

Vitamin A

Another important vitamin involved in vision health is vitamin A. Vitamin A is a general term that refers to a family of fat-soluble compounds that are structurally similar to retinol and share its biological activity. Among these are retinol, α-carotene, ß-carotene, and retinyl palmitate. Vitamin A or carotenoids can be found in dark green leafy vegetables and in vegetables and fruits that are yellow, orange, or red. Vitamin A plays a fundamental role in the retinal response to light. Inadequate vitamin A can result in night blindness, delayed light and dark adaptation, and dry eye (660).

Beyond its essential role in the visual process, vitamin A is directly involved in gene expression, reproduction, embryonic development, and immunity. Vitamin A and ß-carotene serve as biological antioxidants and have been shown in multiple studies to reduce the risk of cancer and coronary heart disease (661, 662). Vitamin A also plays a role, albeit sometimes indirectly, in the function of almost all of the body's organs (663).

Oxidative stress is increased during space flight, and this could affect cardiovascular health and cancer risk, as described in other sections of this book. Vitamin A status may play a critical role in maintaining antioxidant health during space flight; however, as with many antioxidants, the desire to supplement with high doses in the hope of staving off one disease is high, but unwarranted and potentially counterproductive. Excess vitamin A, in levels on the order of twice the recommended daily intake, has been shown to increase bone resorption and fracture risk (271, 664-666). Furthermore, supplementation with ß-carotene should be done with caution (either alone or with vitamin A or in combination with vitamin E), because of unanticipated outcomes of an increased risk of lung cancer in smokers (667, 668). This increased risk among smokers might be related to pro-oxidant actions of ß-carotene in the lung.

When considering pre- and postflight space flight data, there is a significant interaction between the effects of landing site and space flight on serum levels of both retinol and retinol-binding protein (6). Russian landings are different from US landings in that blood samples are usually collected several hours later because of the logistics of the landing site.

Serum retinol decreased from 0.73 ± 0.17 µg/mL to 0.59 ± 0.13 µg/mL when landings were in Russia, and increased from 0.52 ± 0.09 µg/mL to 0.63 ± 0.12 µg/mL when landings were in the US. Similarly, retinol-binding protein decreased from 61.4 ± 5.6 to 50.92 ± 8.41 mg/L when landings were in Russia, and increased from 49.2 ± 9.2 to 53.0 ± 8.7 mg/L when landings were in the US. These differences in landing sites could be related to the time delay in sample collection, the fact that crewmembers might have consumed food during the time delay, or even a difference in the stress response at different sites. These data, however, do not provide evidence that there is a deficiency of any sort for vitamin A.

In-flight vitamin A data have been collected as part of Nutrition experiments on ISS, and Figure 43 shows that no significant changes in retinol or ß-carotene occur during space flight.
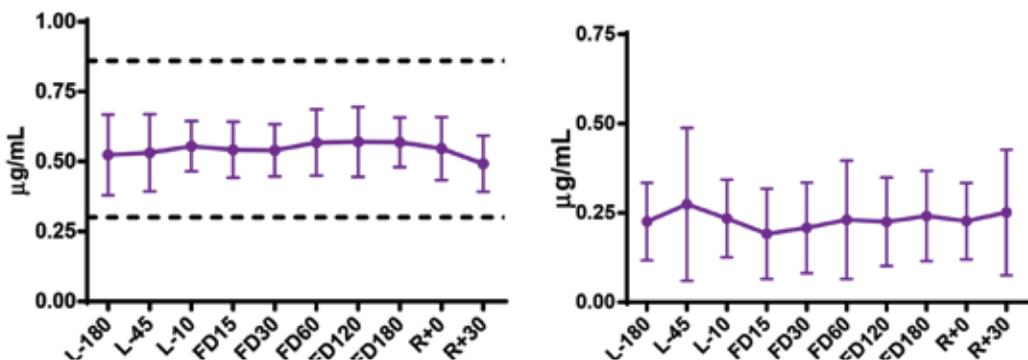
Figure 43. Serum retinol (left) and ß-carotene (right) before, during, and after long-duration space flight. Dashed lines represent normal range. Data are mean ± SD.

In addition to the vision-related changes observed in some astronauts after long-duration space flight, several studies have confirmed that astronauts and cosmonauts have an increased risk of cataract formation after space flight (621, 622, 669, 670). Cucinotta et al (622) identified an increased risk of all types of cataracts (including posterior subcapsular, cortical, and nuclear) among astronauts with greater exposure to radiation. Longitudinal follow-up studies have been conducted and it was determined that progression of cortical cataracts, but not posterior subcapsular or nuclear cataracts, is related to space radiation exposure (620, 621). Although radiation exposure is a large driving force for the oxidative damage that leads to some types of cataracts, the longitudinal study provided evidence that intake of specific nutrients may provide some protective effects (621). In the first report of the NASA Study of Cataract in Astronauts, nutritional intake estimates were obtained from a questionnaire, and the data provided evidence that ß-carotene and lycopene intake had a protective effect for some types of cataracts in astronauts (621). As reviewed by Agte and Tarwadi, numerous ground-based studies have provided evidence for associations between micronutrients and antioxidants (either blood levels or estimated intakes) and cataracts (671). A recent meta-analysis provided similar results supporting an inverse association of α-carotene (and vitamin E, lutein, and zeaxanthin) with age-related cataract (672).

Although epidemiological data support the idea that lower vitamin A status is associated with cataract incidence, it is not known whether altered micronutrient and antioxidant intake during space flight could minimize cataract incidence related to space radiation, as this requires further interventional study and better estimates of in-flight nutrient intake along with nutrient status assessments.

Expedition 35 Flight Engineers Chris Cassidy (pictured) and Tom Marshburn (out of frame) inspect and replace a pump controller box on the International Space Station.

In the International Space Station's Harmony node, European Space Agency astronaut Alexander Gerst, Expedition 40 flight engineer, performs an eye exam for the Ocular Health experiment, which observes and seeks to understand vision changes during long-term space missions.

9 Immune Function, Inflammation, and Nutrition

Optimal function of the immune system is impaired in the presence of malnutrition. Without adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response (673, 674). Nutrients act as antioxidants and as cofactors (675). Historically, during space flight crews generally have lower dietary intake than they do under normal conditions on the ground (2). It is well known from ground research that a lack of macronutrients or selected micronutrients, like zinc, selenium, and the antioxidant vitamins, can have profound effects on immune function (676-679). Such a lack of nutrients also leads to deregulation of the balanced host response (680). Disruption of nutritional balance and dietary intake of astronauts and cosmonauts during space flight, which is often accompanied by a stress response, might influence their immune response (681, 682). However, detailed information on the effects of many micronutrients during space flight are mandatory before specific nutritional recommendations can be made, especially with respect to their relationship with immune system function.

Energy Intake

As discussed earlier, some crewmembers have an insufficient energy intake, which can lead to more extensive free radical propagation because of diminished protein-based antioxidant defense mechanisms (2, 683). On the Mir station and the Life and Microgravity Science Shuttle mission, space travelers who consumed inadequate energy intake had significant increases in urinary excretion of 8-iso-prostaglandin-F2α and 8-oxo-7,8-dihydro-2 deoxyguanosine (8-OH-dG), markers for oxidative damage (684).

Protein and Amino Acids

Stein et al suggest that space flight triggers a stress response similar to the one triggered by stress induced by injury (685). Protein and amino acid deficiencies can have profound effects on a variety of immune system functions (686, 687). With respect to specific amino acids, arginine is necessary for normal T-cell function and may become essential in catabolic states. Supplementary dietary arginine has been shown to have useful effects on cellular immunity in animal studies, showing increased size of the thymus, enhanced lymphocyte proliferation in response to mitogen and alloantigen, augmented macrophage and killer cell lysis, and increased lymphocyte interleukin 2 production and receptor activity (688). Supplementation of arginine led to improved wound healing and immune responses in elderly subjects (689). Judging by these observations it might seem promising to supplement arginine during long-term missions; however, up to now no studies have been carried out to test arginine as a measure to improve immune response during space missions.

Another amino acid beneficial for the immune system is glutamine. Glutamine is the most abundant free amino acid in the body. It can inhibit NF-κB activation and cytokine expression after sepsis (690). Some of the beneficial effects of glutamine are its

antioxidant effects and its actions as a precursor to glutathione, an energy substrate for lymphocytes and neutrophils, and as a stimulator of nucleotide synthesis (691, 692). Glutamine seems to have a significant beneficial effect on mortality, length of hospital stay, and infectious morbidity in critical illness (691). Positive results of glutamine supplementation have been shown in critically ill patients, in whom supplemental glutamine reduced complications and mortality rates in addition to having a stimulating action on the immune system (693, 694). However, up to now supplementation of glutamine as a pharmaconutrient has not been tested in space flight or space flight analogs (eg, bed rest).

Vitamin D

As already described in previous chapters, the classical function of vitamin D is t o regulate calcium homeostasis and thus bone formation and resorption. However, recent publications show that vitamin D also exerts other biological activities including immunomodulation. The latter seems to be mediated by the (nuclear) vitamin D receptor (VDR) expressed in antigen-presenting cells and activated T cells (695). Vitamin D and the VDR are required for the blood to have normal numbers of regulatory T cells. The discovery that VDR is inducible in lymphocytes suggests a role for $1,25(OH)_2D_3$ in the immune system (696). Even the enzyme $25(OH)D_3$-1-α-hydroxylase is expressed by active macrophages, making them able to synthesize and secrete $1,25(OH)_2D_3$ (697). However, in macrophages the enzyme is mainly activated by immune signals such as interferon-ɣ rather than by parathyroid hormone, which is the activator in the kidney (695). Moreover, the active vitamin D metabolite $1,25(OH)_2D_3$ can also be modulated by alternative mechanisms to increase the ability of peripheral blood mononuclear cells from sensitized human donors to resist microbes (here mycobacteria). Martineau et al found that $1,25(OH)_2D$**3** suppressed both bacillus Calmette-Guérin and *Mycobacterium tuberculosis* in infected cell cultures, likely through "nonclassical" mechanisms including the induction of antimicrobial peptides (698, 699). Kondo et al found that vitamin D supplementation improved the sensitivity of the treatment response to pegylated interferon α/ribavirin therapy in chronic hepatitis C patients (700).

Studies during or after space flight have shown numerous changes in astronauts' immune status, including altered distribution of circulating leukocytes, altered production of cytokines, decreased activity of natural killer cells, decreased function of granulocytes, decreased activation of T cells, altered levels of immunoglobulins, reactivation of latent viruses, altered virus-specific immunity, expression of Epstein-Barr virus immediate early and late genes, and altered neuroendocrine responses (701). Furthermore, evidence exists that among individuals wintering over in the Antarctic for 6 months, who have high serum cortisol, a higher vitamin D status is related to a lower probability of viral shedding in saliva (407). In that study an interactive effect occurred between cortisol and vitamin D, and subjects with lower serum 25-hydroxyvitamin D and with the highest quartile of serum cortisol (22.5 μg/dL or higher) had more evidence for shedding of Epstein-Barr virus (EBV) in saliva than did individuals in the lowest quartile of cortisol (13.1 μg/dL or below, Figure 44). Thus, a low vitamin D status of astronauts during space missions might have an impact on their immune status. Further studies are mandatory to distinguish between the effects of vitamin D deficiency and mere microgravity effects.

Figure 44. An interaction between serum cortisol, vitamin D status, and the probability of EBV shedding. Data from all 41 participants in the Antarctic study are included in the graph, and the data were statistically analyzed using the continuous data set of cortisol data. The data are split into the 2 subgroups here for presentation purposes. The graph is from Zwart et al (407).
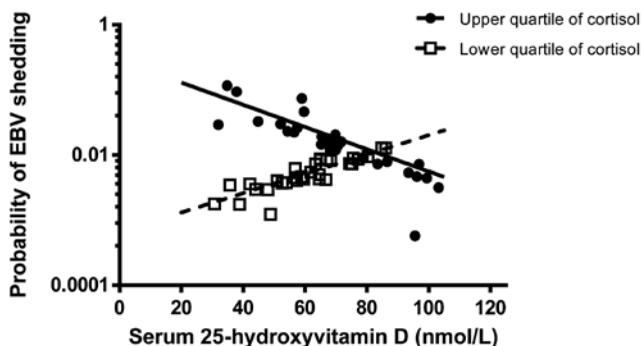
Vitamin B$_{12}$

Vitamin B$_{12}$ functions in many enzymatic reactions, and deficiencies result in anemia, as well as neurological disorders. Vitamin B$_{12}$ functions as a coenzyme in 2 metabolic forms: adenosylcobalamin and methylcobalamin. Vitamin B$_{12}$ works as a cofactor for 3 different enzymatic reactions: (1) the conversion of homocysteine to methionine, (2) the conversion of L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA, and (3) the isomerization of L-leucine and ß-leucine. Vitamin B$_{12}$ deficiency may cause the accumulation of folate in the serum because of a reduction in B$_{12}$-dependent methyltransferase, also known as the methyl-folate trap (648). Vitamin B$_{12}$ also functions in the synthesis of choline, which can be converted to the neurotransmitter acetylcholine. Vitamin B$_{12}$ deficiency may lead to alterations of immunological indicators, such as a reduction of lymphocytes and suppression of natural killer cell activity, both of which can be reversed by supplementation with vitamin B$_{12}$ (702). In one study, for 4 months elderly subjects (aged 70 years) received, in addition to the regular diet, a special nutritional formula providing, among other nutrients, 120 IU vitamin E, 3.8 mg vitamin B$_{12}$, and 400 mg folic acid. NK-cell cytotoxic activity increased in supplemented subjects, indicating increased innate immunity in elderly people (703). These few studies demonstrate the importance of a sufficient B vitamin status to maintain an adequate immune response (704).

Sodium

High sodium intake is correlated with development of hypertension in sodium-sensitive people. We have shown in space flight as well as in ambulatory conditions on Earth that at an intake level of about 4000 mg/d, sodium is retained without being accompanied by fluid retention (30, 537, 705). A hypothesis that might explain how sodium can be bound in an osmotically inactive way has been brought forward by Titze et al (543, 706) and proposes that sodium can be stored on proteoglycans in interstitial sites. This uniquely bound sodium can induce a state of local hypertonicity in the skin interstitium. In a further study they suggest that the local hypertonicity is sensed by macrophages, which then activate a transcription factor (tonicity enhanced binding protein), which in turn induces vascular endothelial growth factor C signaling (538, 539, 707). Macrophages play a key role in innate immunity, and therefore further studies in microgravity should distinguish between the effects of microgravity and high sodium intake on the immune system.

Vitamin A

In brief, vitamin A plays a well-known role in immune function and protection against infections (708-711). A vitamin A deficiency impairs mucosal barriers and diminishes the function of neutrophils, macrophages, and NK cells (712); it may affect host defenses directly (713) or indirectly through its role in epithelial cell differentiation and host barrier function (710). The considerable immunity benefits of vitamin A, which would contribute to reducing the risk of various pathogen-mediated diseases, warrant a recommendation to supplement individuals with minimal or poor vitamin A status. However, whether immunity benefits accrue from providing additional vitamin A to those with sufficient status is not known (714).

Vitamin C

Ascorbic acid (vitamin C) is an essential component of every living cell. The concentration of vitamin C is very high in leukocytes, and the vitamin is used rapidly during infection to prevent oxidative damage. Vitamin C is a regulator of redox and metabolic checkpoints that control activation and survival of immune cells (680). A deficiency in vitamin C status is associated with reduced immune function (715). Vitamin C has been shown to stimulate the immune system by enhancing T-lymphocyte proliferation in response to infection, and increasing cytokine production and synthesis of immunoglobulins (716). However, the antioxidant role during space flight of neither vitamin A nor vitamin C has been investigated up to now. In analog studies such as (short- or long-duration) bed rest, no significant change in vitamin C could be shown (283, 316), but a trend for an increase was apparent. This might be related to dietary vitamin C intake during the study relative to the intake before the study (283).

Vitamin E

Vitamin E is a strong antioxidant that can support monocyte/macrophage-mediated responses (717). Vitamin E and selenium have synergistic functions in tissues to reduce damage to lipid membranes by the formation of reactive oxygen species (ROS) during infections. The ability of vitamin E to scavenge lipid-soluble free radicals depends to some extent on the status of 2 other antioxidant compounds, vitamin C and glutathione, which are involved in reducing oxidized vitamin E back to a reusable (ie, able to be oxidized) form. Additionally, vitamin E may improve T-cell function by decreasing production of prostaglandin E2 by macrophages, by modulating the amino acid cascade initiated by lipoxygenase and/or cyclooxygenase (100). Furthermore, vitamin E influences lymphocyte maturation, possibly by stabilizing membranes and allowing enhanced binding of antigen-presenting cells to immature T cells through increased expression of intercellular adhesion molecule-1.

Copper

Copper has wide-ranging functions in the body, including many considered vital for space flight (77, 502-505). This fact might have direct or indirect (when alterations are induced by psychological stress or radiation stress) implications for nutrition and nutritional status being possible causes or effects of alterations in immune system function (2). Nonetheless, to date, little to no information is available about copper metabolism during space flight.

Zinc

In addition to its many essential functions in growth and development, zinc is essential for the function of cells of the immune system (718). It has an important role in promotion of wound healing and in maintenance of intestinal integrity. A deficiency of zinc is also associated with reduced concentrations of insulin-like growth factor 1 and reduced rates of protein synthesis. Therefore, zinc deficiency could be especially detrimental during immobility. However, zinc status of astronauts, as assessed by mean serum zinc and urinary zinc excretion (admittedly, not the best markers of zinc status), did not change after long-duration space flight (2). No data are available on the use of zinc supplementation as a countermeasure during space flight.

Polyphenols

Naturally occurring polyphenols like resveratrol, quercetin, curcumin, and catechins have shown antioxidant and anti-inflammatory effects (719). These effects seem to be modulated through different pathways such as protein kinase-dependent pathways activated by NF-κB or mitogens, as well as through preventing the generation of reactive oxygen species by iron binding (720). Additionally, polyphenols seem to activate sirtuin 1 directly or indirectly and thereby are beneficial—besides having other functions—for regulation of oxidative stress, inflammation, and autoimmunity. Accumulating evidence has shown that polyphenols such as resveratrol, curcumin, catechins, and quercetins have a regulatory role in immune function in vitro and in vivo (506, 721-726). Therefore, they might also have beneficial effects in prevention of immune dysfunction during long-term space missions, particularly because body iron stores are higher during space flight. However, the role of polyphenols in sirtuin-1–mediated or iron-related regulation in immune function remains to be studied. No results have been obtained during space flight yet; however, a recently selected flight study will examine the role of increasing polyphenol intake on bone health in particular.

Iron

As mentioned previously, the maintenance of iron homeostasis is extremely important for human health. During space flight, it is well established that iron homeostasis is altered (2, 469). The decreased red blood cell mass, increased serum ferritin, decreased transferrin receptors, and increased serum iron all provide evidence for increased iron storage during space flight. Furthermore, the space food system provides almost 3 times the recommended intake (2). Iron plays an ambiguous role in human health: not only do humans require it for survival, but also microorganisms (including pathogens) require iron acquisition from the environment for their survival. Cells of the innate immune system have genes that regulate proteins that can modulate iron homeostasis at the cellular and systemic level to restrict iron availability to invading microorganisms. One such protein is hepcidin, a key regulator of iron homeostasis and a critical factor in the anemia of inflammation (727, 728). Hepcidin has been shown to be endogenously expressed by innate immune cells—macrophages and neutrophils. It plays a role in making iron less available by increasing intracellular iron sequestration and decreasing circulating iron concentrations, and it is influenced by cytokines IL-6 and IL-1 (729, 730). Studies need to be done to determine the role of increased iron stores during space flight on immune function and reactivation of latent viruses.

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs), such as omega-3 fatty acids, protect from oxidative damage and radiation-related risks (99, 731), both of which are concerns for space travelers. The mechanism of action of omega-3 fatty acids on these systems is likely related to multiple pathways, but evidence exists that the nuclear transcription factor NF-κB is affected differently by omega-3 and omega-6 fatty acids (732). This transcription factor affects transcription of genes involved in cell cycle regulation and inflammatory processes. NF-κB is activated by arachidonic acid and specifically by prostaglandin E2, but Camandola and colleagues have found that eicosapentaenoic acid (a PUFA) inhibits NF-κB activation (732).

We have reported elevated NF-κB after short-duration space flight (116). The effects of omega-3 fatty acids on inflammatory cytokines, and specifically TNFα, are well documented on the ground (116, 733-735), but warrant further studies during space flight.

In summary, astronauts in space are generally not optimally nourished. Dietary intake tailored to the astronauts' needs may be beneficial for their immune system function. Furthermore, the environmental stress of space flight can lead to changes in immune response as well as in the nutritional needs of the individual astronaut. Nutrition for optimal immune response and other functions is required to support optimal astronaut health during long-duration missions. However, it is important to be aware that "one size does not fit all." An immune nutrient intake profile that is appropriate for one astronaut or one condition may be of minimal benefit for another individual or condition, and could be harmful in other settings. Making evidence-based decisions in choosing the optimal diet or formula will minimize adverse effects. To reach that level of individualized diet for astronauts, further research is needed to assess whether diet can benefit immune function during space flight. The use of basic clinical pharmacology, molecular biology, and clinical research principles in the study of nutritional therapy during space flight and analog studies will lead to answers on how to administer the right nutrients, in the right amounts, at the right time during astronauts' space missions.

Members of the STS-124 and Expedition 17 crews share a meal in the Zvezda module of the ISS.

10 Antioxidants and Oxidative Stress

The space environment exposes astronauts to numerous sources of oxidative stress. Some of these sources are extravehicular activity (EVA) and EVA prebreathe protocols, exercise, diet, and radiation (469, 472, 736-738). After space flight, anabolic processes and substrate competition between muscle rebuilding and host defense mechanisms stress the antioxidant defense system and may contribute to postflight increases in oxidative damage (683).

Hypoxic Conditions

As exploration missions come closer to reality, we will need to better understand risks associated with the type of EVAs that are currently planned for those types of missions. Current mission designs could involve as many as 30,000 hours of EVA exploration time, which is far more than the 20 total hours spent conducting EVAs during the entire Apollo Program (739, 740). Future vehicles or exploration habitats will likely operate at 8.0 to 8.2 psia and 32% to 34% oxygen with the balance nitrogen (740). EVAs at 4.3 psia would require some amount of oxygen prebreathing. As a comparison, ISS has operated at an Earth equivalent of 14.7 psia and 21% oxygen. The terrestrial altitude equivalent of 8 psia and 32% oxygen is about 6,000 to 8,000 feet (1829 to 2438 m), and would represent a hypobaric hypoxic scenario. Concerns associated with prolonged hypobaric hypoxic conditions include potential increased risk of vision impairment issues, acute mountain sickness, sensorimotor impairment, alterations in cardiovascular and immune function, and anorexia.

Extravehicular Activity

Extravehicular activity (EVA), or spacewalking, is a unique situation from a nutritional perspective, because the EVA suit does not easily allow food consumption. On early Shuttle missions, a 165-kcal fruit bar was custom-made to fit in the EVA suit, but it was typically not consumed, and is no longer included. Crewmembers can go without food for as long as 8 to 10 hours while they are preparing for and performing EVA. Nutritional recommendations for EVA were designed to help maximize crew performance and efficiency. When nutrition for EVA was reviewed in 1991, the recommendation for EVA crewmembers was that they should consume an additional 500 kcal on days of EVA (741). This was designed to account for the metabolic cost of EVA (~200 kcal/h).

In 2000, another review of this situation was requested by NASA's Flight Medicine Division. The resulting recommendation was to provide food items for consumption during EVA preparation (as close as possible to the donning of helmets). The food items should contain 300 to 500 kcal, with about 70 to 100 g of carbohydrate and a high content of soluble fiber. Candidate items are reviewed to ensure that, in the attempt to meet the basic criteria, undesirable nutrients or additives are not included, and that crew preferences are accounted for. It was also recommended that crewmembers reconsider use of the in-suit food bar, or that alternatives be sought.

Fluid intake during EVA is also a topic of concern. Crewmembers lose 6 to 8 ounces (177 to 237 mL) of fluid per hour during an EVA. The current EVA suit contains a 24- or 32-ounce (710 or 946 mL) drink bag. Only water is used (early EVAs included flavored beverages, but a problem during a lunar EVA resulted in a programmatic decision to include only water). Provision of in-suit fluid is an important factor in suit design. For the current suit, use of the 32-ounce drink bag is recommended. The development of a larger, disposable drink bag is highly encouraged. The disposable drink bag should be designed so that a flavored drink (such as the current Shuttle food system beverages) could be used to increase palatability and intake, assuming that the technical concerns can be eliminated.

Because missions to explore a non-Earth surface (planetary or asteroid) will likely include EVAs similar in duration to current ISS EVAs (8 to 10 h) but more frequent (2-3 times per week, instead of 2-4 times/6 months), a clear need exists for development of nutrition support during EVAs. A nutrition support system will need to fit the lunar suit design. The definition of the optimal nutrition support system will need to be based on the results of ground studies designed to optimize performance, minimize fatigue, and minimize oxidative damage from a high partial pressure of oxygen in the suit.

Reactive Oxygen Species and Exercise

Exercise-induced fatigue and muscle atrophy are mediated in part by reactive oxygen species (ROS). Electron spin resonance spectroscopy technology confirmed earlier findings from the 1950s suggesting that short-lived reactive intermediate molecules like ROS are present in skeletal muscle after exercise (742). Since then, numerous studies have supported a role of ROS in skeletal muscle fatigue (742-744). Mitochondria are the major source of ROS in muscle cells, where a fine balance of ROS exists between maximizing force and minimizing fatigue (736). Antioxidant-mediated depletion of ROS from unfatigued muscle yields decreased production of skeletal muscle force (745). On the other hand, excess ROS can be detrimental in terms of fatigue. ROS can denature proteins directly associated with the sarcoplasmic reticulum Ca^{2+} release mechanism (746), thus compromising tension development. Also, rat studies show that xanthine oxidase-induced ROS yields increased diaphragm fatigue, and that the elevated ROS during intense exercise is implicated in the onset of muscle fatigue (747). Furthermore, decreased antioxidant status lowers exercise capacity and increases onset of fatigue in human and animal studies (742, 744).

Astronauts perform prolonged upper-body exercise during EVA activity, and one of the limiting factors in completing EVA tasks is forearm and hand muscle fatigue due to extensive tool operation. The fatigue often requires crewmembers to stop and rest, thereby prolonging the duration of EVA, and limits the number of tasks performed during each EVA. To date, there is little evidence showing that antioxidant supplementation has a benefit for improving muscle performance and inhibiting fatigue. Given the nature of the requirement for homeostasis of redox systems, there is a potential for antioxidant overload to decrease muscle force potential instead of having a protective effect.

Radiation Exposure

Astronauts are exposed to highly ionizing radiation, in addition to secondary radiations resulting from interactions of ionizing radiation with shielding materials or the human body. Biological effects of radiation include damage to DNA from a direct hit from an ion track, oxidative damage from generated ROS, and oxidative damage induced

by a bystander effect (99, 748, 749). A bystander effect occurs when cells damaged by radiation particles secrete cytokines or other proteins that can generate ROS in cells that are not destroyed (750).

Oxidative Damage Markers During Space Flight and in Ground Analogs

Evidence for oxidative stress resulting from space flight exposure exists in multiple tissues, including ocular tissue (670, 751), urinary and blood biomarkers of damage to DNA, lipids, and protein (6, 469, 684, 752), and in gene expression (753, 754). Plasma malondialdehyde (MDA), 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and urinary 8-hydroxy-2'-deoxyguanosine (8OHdG) have been measured during and after flight as indicators of lipid peroxidation (MDA and $PGF_{2\alpha}$) and DNA damage (8OHdG) (4, 684). A significant elevation of urinary 8OHdG has been noted after long-duration missions (Mir and ISS) (6). These data are supported by results from the ground-based analog NEEMO, in which crewmembers underwent 10- to 14-day saturation dives (476, 755). Urinary $PGF_{2\alpha}$ was significantly decreased during flight but elevated about 2.5-fold after flight (684), and plasma MDA was increased both during and after flight (684). In a Russian 120-day bed rest study, increased concentrations of markers of lipid peroxidation were found in subjects, and this increase was mitigated with vitamin E (756).

The apparent increases in oxidative damage observed during and after flight could be caused by a number of factors, including altered DNA repair mechanisms, decreased antioxidant defense systems, or simply increased oxidative stress. Microgravity does not affect the repair of double-strand chromosome breaks (757, 758), but evidence exists that downregulation of antioxidant defense systems occurs during space flight (759). Along with increases in markers of oxidative damage and decreases in antioxidant defense systems, a decrease in total antioxidant capacity also occurs.

Antioxidants and Related Nutrients: Selenium, Vitamin E, Vitamin C

Selenium has been shown to play a role in the maintenance or induction of cytochrome P450, pancreatic function, DNA repair, enzyme activation, immune system function, and detoxification of heavy metals (760). Selenium is also a cofactor for glutathione peroxidase, which plays a role in the reduction of organic peroxides and hydrogen peroxide. Selenium has also been shown to be necessary for iodine metabolism.

Postflight reductions in serum selenium of more than 10% have been observed after ISS flights (6). Whether this is related to intake or metabolism is not known.

Deficiency of selenium can lead to impaired immune function, illness (Keshan disease and Kashin-Beck's disease), or even death. Excess selenium can lead to problems affecting gastrointestinal, neurological, cardiopulmonary, and renal systems (760). Toxicity is not likely to occur except when selenium is consumed in large amounts in dietary supplements, but care must be taken to avoid toxicity despite the relationship of selenium to cancer risk and antioxidant status.

Vitamin E is a lipid-soluble, chain-breaking antioxidant found in body tissues, and is also the first line of defense against lipid peroxidation reactions. Eight naturally occurring compounds have vitamin E activity: 4 tocopherol derivatives (α-, γ-, δ-, and ß-tocopherol) and 4 tocotrienol derivatives (α-, γ-, δ-, and ß-tocotrienol) (761). The tocopherols that are most abundant in biological systems are α- and γ-tocopherol, but small amounts of δ-tocopherol and γ-tocopheryl quinine are also present. About 90% of the tocopherol found in human plasma is in the form of α-tocopherol (762). After ISS crewmembers had spent 4 to 6 months in space, their plasma γ-tocopherol was 50% less than preflight levels (6). No change in α-tocopherol occurred in these subjects.
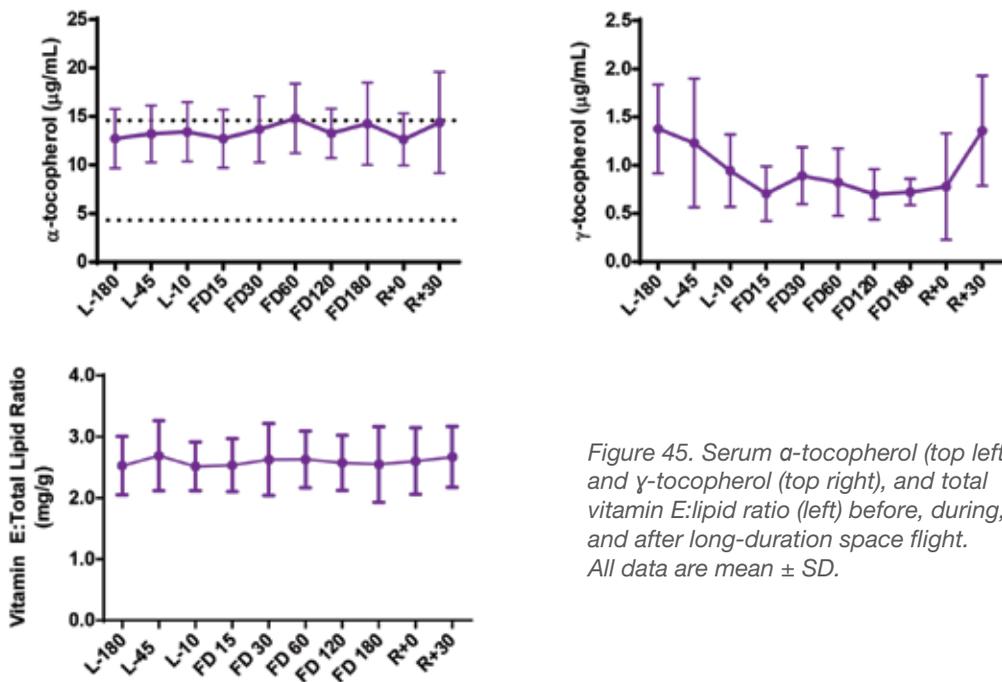
Figure 45. Serum α-tocopherol (top left) and γ-tocopherol (top right), and total vitamin E:lipid ratio (left) before, during, and after long-duration space flight. All data are mean ± SD.

Oxidative stress can increase in microgravity and high-radiation environments (683, 684, 763), and the antioxidant properties of vitamin E may help to counteract the free-radical damage caused by high-linear energy transfer radiation in space. Pretreatment with antioxidants may help decrease radiation damage during missions (764), and it may be necessary to provide enough vitamin E for astronauts' blood levels of the vitamin to be higher during space flight than on Earth. However, knowledge gaps weaken the evidence for use of vitamin E as a countermeasure. Clinical trials have documented negative side effects of pharmacologic vitamin E supplementation alone or with other antioxidants; it can increase risks of cancer in humans and animals (765-768).

The term "vitamin C" actually refers to 2 different compounds, ascorbic acid and dehydroascorbic acid, both of which have activity against scurvy (769). Vitamin C functions as an antioxidant because it acts as a reducing agent for most physiologically relevant reactive oxygen species, reactive nitrogen species, singlet oxygen, and hypochlorite. It serves as a cofactor for enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters (769). Vitamin C also provides antioxidant protection by returning α-tocopherol to its biologically active state during lipid oxidation. The reducing agents glutathione and either reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) regenerate the oxidation products of ascorbate (769).

It has been suggested that vitamin C requirements should be greater for persons who are under excessive physical or emotional stress, given the role of ascorbate in the biosynthesis of steroid hormones and neurotransmitters. However, no substantial data show that vitamin C metabolism is altered in healthy subjects under mental or emotional stress (762).

As a cofactor in collagen synthesis, vitamin C has been investigated for potential effects on bone health. Although in the Framingham Study higher vitamin C intake was found to be associated with lower bone mass (770), the significance of this association was marginal when data were adjusted for potassium intake. This suggests that vitamin C

may be a secondary factor in bone loss, related to fruit and vegetable reduction (274, 771-773), as described elsewhere in this text. Other studies have found that vitamin C intake or supplement use is related to improved bone health and bone mass, but this relationship depends on adequate calcium intake (774-777). Vitamin C has been related to cataract and cancer incidence (778-780), both of concern for space travelers.

Deficiency of vitamin C leads to fatigue, depressed immune function, scurvy (fatigue, muscle cramps, bruised and/or bleeding gums), and eventually even death. As noted in the introduction, scurvy resulted in more sailor deaths during the age of sail than all other causes of death combined (1). Toxicity of vitamin C leads to gastrointestinal distress, and has been reported in subjects consuming more than 1000 mg/d (778).

Vitamin C assessments of ISS crewmembers have been conducted, with generally no changes after landing relative to before launch (unpublished data). Recent long- and short-duration bed rest studies documented no statistically significant change in vitamin C, but results showed a trend for an increase, which might be related to dietary intake during the study relative to the subjects' nominal intake (134, 316).

The stability of vitamin C in food supplies has been studied, and it is generally unstable at a neutral or alkaline pH and in high-oxygen environments (781). Vitamin C is also unstable when exposed to light or heat (781), and in irradiated foods (782, 783). Salem (783) found that gamma irradiation of fresh onion bulbs significantly reduced their vitamin C content. This group also found that vitamin C content of onion bulbs had decreased about 50% after 6 months of storage. The destructive effects of gamma irradiation (10 kGy) on vitamin C were also evident in commercial spices such as basil, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage (784). Exposure of these spices to gamma rays for >3 months resulted in a marked increase in the concentration of quinone radicals. Evaluation of vitamin C stability in space foods showed an average loss of 27% after 1 year of storage (785). Fortunately, the vitamin C content of food items and potential menus is high enough that, depending on mission profile, intake of vitamin C might still meet requirements (785).

A major concern for space flight is the possibility that vitamin C could be degraded in foods during extended-duration missions when space foods are exposed to large amounts of radiation and undergo long-term storage (foods may be sent to Mars in advance of the crew, and left there for up to 5 y). This could be catastrophic.

Free-radical formation is increased in space because greater amounts of radiation are present than on Earth. Because of this and increases in other oxidative stressors, antioxidants such as vitamin C are in greater demand by the body to act as buffers and minimize the oxidative damage. Studies have shown that supplementation with vitamin C and other antioxidants can modify human tissue radiosensitivity and protect DNA against damage (786, 787). Just as important to consider, however, is the possibility that vitamin C could induce DNA damage. Cai and colleagues (787) found that vitamin C can act as an antioxidant to prevent DNA damage caused by ionizing radiation, but in the presence of copper, it can also act as a reducing agent to induce DNA damage. Because vitamin C can reduce redox-active metals such as iron and copper, this "antioxidant" can increase the pro-oxidant chemistry of these metals (788). Thus, vitamin C can serve as both a pro-oxidant and an antioxidant, and the amount of it required by exploration crewmembers needs to be carefully addressed (as does the amount of almost all nutrients).

Vitamin C content and stability in the space food supply need to be determined. Evaluation of the impact of vitamin C supplementation during exposure to oxygen or high-linear energy transfer radiation should be investigated before recommendations

can be made for supplement use during flight. This should be evaluated in a coordinated effort to find an antioxidant profile for space travelers. After data have been gathered about vitamin C status during and after flight, and preferably after data are available pertaining to the influence of space flight-induced stress on vitamin C, an evaluation of intake requirements needs to be made.

Expedition 16 Commander Peggy Whitson pauses for a photo while working on the S5 Truss during Bearing Motor Roll Ring Module removal and replacement operations.

11 Pharmacology and Drug-Nutrient Interactions

Supplements versus Whole Foods

The benefits of supplements are such that individual nutrients can be obtained when the diet cannot meet needs. Drawbacks to supplements include the potential for side effects, interactions with medications, and, with respect to bone, the fact that isolated nutrients may not provide the same protective effect on bone as they would in the matrix of the whole food. For example, more than 100 phytochemicals in tomatoes likely contribute to the chemoprotective effect of tomato puree in addition to the lycopene known to protect against certain types of cancers. Tomato puree has much stronger dose-dependent, antimutagenic effects and lowers biomarkers of oxidative stress more than pure lycopene alone (789, 790). Similarly, omega-3 fatty acids have different effects on vasodilation, depending on whether they are supplied as a supplement or in a whole food (791, 792).

Besides the fact that supplements lack the synergistic effects of nutrients in whole foods, there are numerous examples of negative side effects associated with supplement use. Symptoms can range from gastrointestinal effects, dizziness, or decreased white blood cell count (from ipriflavone, which is synthesized from the soy isoflavone daidzein) to increased cancer risk (ß-carotene in the CARET study) or increased risk of stroke (667, 793-795).

Provision of nutrients through supplements also ignores the fact that in some cases, for example the omega 3:omega 6 ratio, the negative effects of other foods cannot be overcome simply by provision of supplements. Although this phenomenon is more difficult to document, it is likely the reason that epidemiological data continue to show benefits of dietary patterns over supplements.

The issue of supplement use often arises with discussion of nutrient requirements for space travelers and the use of nutrients as countermeasures to the negative effects of space flight, especially oxidative damage and radiation-induced cancer risk. It is generally agreed that nutrients should be provided to astronauts in standard foods instead of supplements (35, 425, 426, 741, 796). The need for more detailed information about the "psychophysiology of hunger and eating" was noted decades ago during the early space programs (8), but this topic has yet to be studied in detail. It is clear from astronauts' experiences on the Mir that when humans are in an isolated environment far from home, food becomes a psychological factor that can be a source of support or a source of frustration.

NASA currently does not recommend that astronauts take general nutritional supplements during flight, for several reasons. Experience to date indicates that crewmembers do not consume the recommended amount of energy intake, and accordingly, intake of many individual nutrients is therefore also inadequate. Unfortunately, the concept of a vitamin and mineral supplement to remedy this is unwarranted, as the primary problem—inadequate intake of food/energy—will not be resolved by a supplement. This situation may even be worsened if crewmembers believe that taking the supplement reduces the need for adequate food consumption, and thus eat even less. Furthermore, when many nutrients are provided as oral supplements,

they are not metabolized by the body as they are when in foods (797). Changes in bioavailability and metabolism of nutrients can increase the risk of malnutrition.

Vitamin or mineral supplements should be used only when the nutrient content of the nominal food system does not meet the requirements for a given nutrient, or when data show that the efficacy of single (or multiple) nutrient supplementation is advantageous. To date, 1 supplement has met this standard, and that is vitamin D. Vitamin D supplements have been provided to all US crewmembers on ISS. Early crews received 400 IU vitamin D_3 per day (6), but recently this was raised to 800 IU per day (425); this level allows maintenance of serum 25-hydroxyvitamin D around 75 nmol/L (31).

Before a supplement is recommended, a clear deficit of that nutrient in the space food system must be identified, as was the case with vitamin D. Stability of nutrients in the form of supplements would also need to be addressed; shelf lives for exploration travel must be particularly long. Supplements, if they are recommended, would need to be tested in ground models for their efficacy in maintaining nutrient status, their stability over a long duration (3 to 5 y), and their potential interaction with pharmaceuticals. Most importantly, supplements will need rigorous testing to demonstrate that the level used is not toxic to other body systems, and will need close monitoring during flight to ensure that their interactions with the space flight environment do not prevent them from being effective or safe. For example, ground-based studies have shown that high doses of antioxidants, when provided in situations where oxidant stressors are present (such as cigarette smoking), can actually have a detrimental effect (798). Recent studies have also found that supplementation with certain antioxidants such as vitamin E and vitamin A can increase risks of cancer and all-cause mortality (767, 768).

An understanding of interactions between nutrients and drugs used in medical care is necessary to implement safe and effective medical care and clinical intervention operations for astronauts on long-duration missions. The most common studies of nutrient-drug interactions concern their effects on a nutrient's or drug's absorption, distribution, biotransformation, and excretion.

Normally, drugs must undergo biotransformation to allow their activation or excretion. For the activity of a drug to be terminated by excretion, the compound must be made water-soluble by biotransformation. For most drugs, this process yields a water-soluble compound that is less active than the original compound. Biotransformation occurs in 2 phases. Phase I is an oxidation or hydrolysis reaction to expose, add, or cleave a functional group. Cytochrome P450 enzymes are involved in this process. Humans have 12 families of cytochrome P450 enzymes, but CYP1, CYP2, and CYP3 are the forms most commonly used in drug metabolism (799). Cytochrome P450 enzymes are unique in their ability to use a wide range of substrates (800). Phase II biotransformation involves the conjugation of the parent compound to a polar group (acetate, glucuronides, sulfates, amino acids, glutathione), which inactivates most drugs. Biotransformation of drugs is influenced by several factors that could be affected by space flight and the space food system: dietary factors, nutrient metabolism, monoamine oxidase inhibitors, and antacids and proton pump inhibitors.

Dietary Factors

Dietary factors (either excess or deficiency) can influence both phases of drug biotransformation. In phase I, 3 factors are required: a sufficient energy source (because of the high energy demands of this system), a protein source for enzyme synthesis, and iron for cytochrome formation (801). Phase II requires glucose, sulfur-containing amino acids, and glutathione (801).

The effects of nutrients on drug metabolism have been well studied in animal models; however, relatively few dietary factors have been studied in humans (801, 802). Results from animal studies must be carefully weighed because of some differences between the cytochrome P450 enzymes of animals and humans.

One of the most well-documented food-drug interactions is between grapefruit juice and a number of medications (803, 804). Flavonoid compounds such as naringin, naringenin, limonin, and obacunone, which are present in grapefruit juice, act as substrates for particular intestinal cytochrome P450 enzymes (CYP3A4 and CYP1A2). Within hours of ingestion, grapefruit juice decreases CYP3A4 protein expression for up to 24 hours (805, 806). The decrease in CYP3A4 is associated with a decreased capability for drug metabolism, and therefore increased drug bioavailability.

Other foods, nutrients, or supplements known to affect phase I and II biotransformations and cytochrome P450 enzymes include protein, carbohydrates, lipids, certain vitamins, minerals, char-broiled foods, red wine, monosodium glutamate and aspartate, and herbs such as St. John's wort (801, 802, 807-810). Generally, high-protein diets increase drug metabolism, and low-protein diets decrease drug metabolism. For instance, antipyrine and theophylline are metabolized more rapidly when subjects are on a high-protein diet (802). Other macronutrients, including carbohydrates, can affect phase I and phase II biotransformation reactions when intakes are very high or low. Theophylline (for asthma) is particularly sensitive to dietary protein:carbohydrate ratios; increasing the ratio can decrease effectiveness of the drug, and decreasing the ratio may lead to toxicity of the drug (811). Fatty acids in the diet can also affect cytochrome P450 enzymes because they can be metabolized by these enzymes. Specifically, CYP2E1 is responsible for lipid peroxidation, and activity of this enzyme is enhanced in the presence of highly polyunsaturated fatty acids such as fish oils.

Metabolism of Nutrients

Some nutrients are metabolized by cytochrome P450 enzymes; therefore, drugs or other nutrients that alter the activity of these enzymes can alter nutrient metabolism. Vitamin D and vitamin A are 2 examples of nutrients whose metabolism involves cytochrome P450 enzymes.

Exposure of 7-dehydrocholesterol to sunlight converts this substrate to previtamin D_3. Previtamin D_3 undergoes an isomerization to form vitamin D_3, a biologically inactive compound. CYP27A is a mitochondrial mixed-function oxidase that is responsible for hydroxylating vitamin D_3 to form 25-hydroxyvitamin D_3 (812). CYP3A4 has been found to be a 25-hydroxylase as well (813). CYP27B converts 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. CYP24 is a 24-hydroxylase that hydroxylates the vitamin D side chain and ultimately terminates hormonal activity of the vitamin. Inhibition of CYP24 has recently been targeted in the development of novel anti-cancer drugs. Because 1,25-dihydroxyvitamin D_3 exerts antiproliferative and differentiating effects on many cell types including cancer, preventing its inactivation by inhibiting CYP24 activity may prove to be beneficial in treating cancer (814). Certain drugs are known to activate CYP24 activity, including rifampin, isoniazid, and phenobarbital (815, 816). Several studies show a relationship between the use of these drugs and osteomalacia (817, 818), which is caused by a deficiency of vitamin D. The discovery of the involvement of CYP3A4 in the metabolism of vitamin D may explain the effects on vitamin D metabolism of numerous drugs, including inducers or inhibitors of this enzyme (for example, grapefruit juice, erythromycin, omeprazole, carbamazepine, and dexamethasone), or implicate them in unexplained effects on vitamin D metabolism.

Vitamin A metabolism involves the actions of CYP1A2 and CYP4A4 in the conversion of retinol to retinoic acid (819, 820). Inducers of CYP1A2 (cigarette smoke, cruciferous vegetables, broiled beef, rifampin) may affect vitamin A metabolism.

Monoamine Oxidase Inhibitors

First-generation monoamine oxidase inhibitors include agents such as antidepressants (phenelzine, tranylcypromine, pargyline, and selegiline), chemotherapeutic drugs (procarbazine), antiprotozoal drugs (furazolidone), and analgesics (meperidine). Monoamine oxidase is responsible for metabolizing dietary phenylethylamines, including tyramine, in the gastrointestinal tract and in the liver. Inhibitors of monoamine oxidase prevent the breakdown of these compounds, and therefore the compounds are taken up in the brain. In the brain, tyramine displaces norepinephrine from storage vesicles, which results in release of a flood of norepinephrine at synapses. Acute hypertension and the potential for stroke or myocardial infarction could result from this process (801). Fermented foods and protein-rich foods that have begun to spoil are rich in phenylethylamines (801).

Antacids and Proton Pump Inhibitors

By altering the pH of the stomach, chronic antacid or proton pump medications can negatively affect the bioavailability of several nutrients, including phosphate, thiamin, folate, vitamin B_{12}, vitamin C, iron, and vitamin A (801, 821, 822). Antacids can precipitate folic acid at a pH greater than 4.0, thus rendering it insoluble and not available for absorption (823). A high pH also affects thiamin bioavailability because the vitamin is not stable at high pH (801). Similarly, at a neutral pH, the antioxidant action of vitamin C on dietary nitrites is hindered. Normally, dietary nitrite is quickly reduced to nitric oxide by ascorbic acid in the acidic gastric juice and it is then absorbed by the mucosa. However, at neutral pH, the nitrite does not react with ascorbic acid and instead accumulates in the stomach, which can increase the likelihood that potentially carcinogenic N-nitroso compounds will be formed (822). These changes are observed mostly in subjects who are infected by *Helicobacter pylori* and are taking proton-pump inhibitors (822).

Vitamin B_{12} and vitamin A are also malabsorbed at higher pH because an acidic environment is essential for their release from dietary proteins. Because large stores of vitamin B_{12} exist in the body, malabsorption of this vitamin is unlikely to lead to deficiency unless a subject has been taking proton pump inhibitors chronically for at least 2 years (821). This would be particularly harmful if vitamin B_{12} stores were low before initiation of therapy.

Summary of Pharmacology and Drug-Nutrient Interactions

Currently no data are available that pertain to specific drug-nutrient interactions during space flight. The main concerns for a long-duration mission involve use of pharmacological agents that are taken chronically. Side effects will be especially harmful if the status of all nutrients is not adequate at the beginning of a long-duration mission. Addressing these concerns of drug-nutrient interactions will be especially crucial for crewmembers who embark on exploration-class missions lasting several years.

12 Summary

As we write this section of the book in the spring of 2014, the Space Shuttle has been retired for a few years, the International Space Station (ISS) has blossomed into an incredible science platform, commercial vehicles are delivering cargo to the ISS (and in the coming few years they will transport crews to and from ISS as well), preparations are underway for the first 1-year stay on ISS, and NASA is targeting development of a vehicle that will allow exploration beyond low Earth orbit, destination at this point TBD—to be determined. These exploration-class missions will require a food system with items having even longer shelf lives than those currently available for the ISS missions (56, 824). As NASA designs the vehicles for these missions, the challenges for the food system will be very similar to those met by all previous space food systems. Mass and volume of the food system and its associated packaging will need to be limited. Refrigerators and freezers will most likely not be available. Acceptability of the food items will become even more important on a multi-year mission. New challenges for this exploration food system will include the need for a 3- to 5-year shelf life and the possibility that the increased radiation encountered on a trip to Mars might affect the nutritional content and quality of the food over time.

Long-term plans for exploration will include the establishment of habitats, which will no doubt include the growing of plants to aid in the recycling of air and water within the habitat (825). These crops will be available for use in the food system. The presence of partial gravity will allow crops to be processed into ingredients (for example, milling wheat into flour) and then used to prepare menu items for crew consumption (56). The research to support these endeavors, especially the growing of crops that will sustain a crew and not just supplement meals made on Earth, still has some distance to go.

These long-term missions will require careful planning of nutrition. Understanding nutrient requirements and utilizing the food system to fulfill them will allow mitigation of some of the negative effects of microgravity on human physiology. Even a marginal nutrient deficiency over a long enough period could be devastating. After the requirements are defined and we have a detailed understanding of absorption, metabolism, and excretion of each nutrient, provision of these nutrients and an understanding of their stability in the space environment (for the months to years before they are consumed) will be critical.

Nutrition is essential for health—on Earth and in space. Determining the nutritional requirements for travelers on short-, medium-, and long-duration exploration missions will be crucial for ensuring crew health and safety, during the mission and after their return. Only the surface has been scratched in our understanding of the ability of nutrients and nutrition to mitigate negative effects of space travel, but the initial results are promising already, as documented throughout this text. In many ways, nutrition offers a suite of countermeasures that require no more crew time than that already allotted for meals. While care clearly needs to be taken to avoid excess amounts of any nutrient, the risks of using nutritional countermeasures relative to those of using pharmacological countermeasures are negligible.

This document details the evidence collected to date that shows why inadequate nutrition is a risk during long-term space travel. The evidence is substantial, and drives a significant ongoing effort to understand and optimize nutrition for space travelers and to use nutrition as a tool to mitigate the health risks of microgravity exposure. Just as for the sailors who left Europe in sailing ships, it is not enough to have food; one must have the right food.

13 Appendices

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SMS, SRZ, MH.

Nutrient Intake Data

Updated nutrient intake data for several space programs are reported below. For ISS, we report the data on nutrients available from the Food Frequency Questionnaire analysis. Data on planned ISS menu content and information on a wider range of nutrients are available online at http://go.nasa.gov/QS1KW1 (2).

	Apollo	Skylab	Shuttle	ISS (E1-13)	ISS (E14-25)	ISS (E26-37)
N	33	9	32	19	19	17
Energy, kcal/d	1880 ± 415[a]	2897 ± 447	2090 ± 440	2313 ± 514	2317 ± 591	2444 ± 536
Energy, % WHO	64.2 ± 13.6	99.1 ± 8.2	74.2 ± 16.0	79 ± 18	83 ± 17	84 ± 15
Protein intake, g/d	76.1 ± 18.7	111.0 ± 18.4	78.0 ± 18.8	102 ± 25	96 ± 34	109 ± 30
Protein intake, % of kcal	16.3 ± 2.1	15.7 ± 2.1	14.9 ± 2.4	18 ± 2	16 ± 2	18 ± 2
Carbohydrate intake, g/d	268.9 ± 49.1	413.3 ± 59.3	304.0 ± 67.3			
Carbohydrate intake, % of kcal	58.1 ± 7.1	57.5 ± 9.1	58.4 ± 5.0			
Fat intake, g/d	61.4 ± 21.4	83.2 ± 13.8	64.0 ± 17.8			
Fat intake, % of kcal	28.9 ± 5.5	26.8 ± 8.6	27.2 ± 4.4			
Calcium, mg/d	774 ± 212	894 ± 142	826 ± 207	878 ± 274	944 ± 258	1074 ± 205
Phosphorus, mg/d	1122 ± 325	1760 ± 267	1216 ± 289			
Magnesium, mg/d		310 ± 58	294 ± 74			
Iron, mg/d			15.0 ± 3.9	18 ± 5	18 ± 5	20 ± 5
Zinc, mg/d			12.0 ± 2.9			
Sodium, mg/d	3666 ± 890	5185 ± 948	3984 ± 853	4601 ± 1239	4658 ± 1593	3823 ± 785
Potassium, mg/d	2039 ± 673	3854 ± 567	2391 ± 565	3315 ± 513	3214 ± 863	3559 ± 784
Water, g/d	1647 ± 188[b]	2829 ± 529	2223 ± 669	2012 ± 462	2142 ± 387	2320 ± 581

Abbreviations: E, expedition numbers of ISS missions; ISS, International Space Station; WHO, World Health Organization.

[a] All data are mean ± SD. Empty cells show where data were not available. Data updated and expanded from earlier reports (2, 826).

[b] n=3 for water intake during Apollo missions.

List of Figures

Overview of 1-carbon metabolism. AA, amino acids; CBS, cystathionine ß-synthase; CYS, cystathionine; FA, fatty acids; HCY, homocysteine; αKBT, α-ketobutyrate; MCA, methylcitric acid; MM-CoA, methylmalonyl coenzyme A (CoA); MMA, methylmalonic acid; MS, methionine synthase; 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTRR, 5-methyltetrahydrofolate homocysteine methyltransferase reductase; PRP-CoA, propionyl CoA; SAH, S-adenosylhomocysteine; SAM,S-adenosylmethionine; SUC-CoA, succinyl CoA; THF, tetrahydrofolate

Artistic depiction of vision issues related to space flight, and the potential involvement of genetic influences in this problem. The depiction of a different DNA base pair (blue above and red below) in the 2 strands reflects genetic polymorphisms, or multiple forms, of the DNA. Inset imagery includes ISS and a Hubble Space Telescope image of the Cat's Eye Nebula, to reflect that these issues are relevant for not only ISS missions, but exploration-class missions as well.

RBC (left) and serum folate (right) before, during, and after long-duration space flight (data are mean ± SD). Note: RBC folate data are not available during flight because of sample processing requirements.

Serum retinol (left) and ß-carotene (right) before, during, and after long-duration space flight. Dashed lines represent normal range. Data are mean ± SD.

An interaction between serum cortisol, vitamin D status, and the probability of EBV shedding. Data from all 41 participants in the Antarctic study are included in the graph, and the data were statistically analyzed using the continuous data set of cortisol data. The data are split into the 2 subgroups here for presentation purposes. The graph is from Zwart et al (407).

Serum α-tocopherol (top left) and ɣ-tocopherol (top right), and total vitamin E:lipid ratio (left) before, during, and after long-duration space flight. All data are mean ± SD.

Abbreviations

8OHdG	8-hydroxy-2'-deoxyguanosine
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ARED	Advanced Resistance Exercise Device
BR	bed rest (day)
BSAP	bone-specific alkaline phosphatase
cal	calorie
CoA	coenzyme A
CO_2	carbon dioxide
d	day, deci
DLR	German Aerospace Center
DNA	deoxyribonucleic acid
DRI	dietary reference intake
DXA	dual-energy x-ray absorptiometry
EBV	Epstein-Barr virus
EE	energy expenditure
Eq	equivalent
ESA	European Space Agency
EVA	extravehicular activity (space walk)
FDx	flight day x
FFQ	Food Frequency Questionnaire
g	gram
g	acceleration due to gravity (1*g* = Earth gravity)
GLA	gamma-carboxyglutamic acid
Gy	Gray
h, hr	hour
HRP	Human Research Program
Hz	hertz
IOM	Institute of Medicine
iRED	interim resistance exercise device
ISS	International Space Station
IU	international unit
J	joule
k	kilo
KCit	potassium citrate
KMgCit	potassium magnesium citrate
L-x	x days before launch
L	liter
LBNP	lower-body negative pressure
μ	micro
m	meter, milli
M	mega
MDA	malondialdehyde
MELFI	minus-eighty (degrees) laboratory freezer for ISS
mol	mole

MRI   magnetic resonance imaging
n   nano
n   number of subjects in a subsample
N   number of subjects in a sample of a population
NAD   nicotinamide adenine dinucleotide
NADH   reduced form of nicotinamide adenine dinucleotide
NADP   nicotinamide adenine dinucleotide phosphate
NADPH   reduced form of nicotinamide adenine dinucleotide phosphate
NASA   National Aeronautics and Space Administration
NBL   Nutritional Biochemistry Laboratory
NEEMO   NASA Extreme Environment Mission Operations
NF-κB   NF-kappa B
NK   natural killer
NTX   n-telopeptide
P   probability, phosphate
PGF2α   8-iso-prostaglandin F2α
PL   pyridoxal
PLP   pyridoxal 5'-phosphate
PM   pyridoxamine
PMP   pyridoxamine 5'-phosphate
PN   pyridoxine
PNP   pyridoxine 5'-phosphate
Post   after bed rest
Pre   before flight or bed rest
psia   pound(s) per square inch absolute
PTH   parathyroid hormone
PUFA   polyunsaturated fatty acid
r   bivariate correlation coefficient
R+x   x days after landing (recovery) or end of bed rest
RBC   red blood cell
RDA   recommended dietary allowance
RNA   ribonucleic acid
ROS   reactive oxygen species
SD   standard deviation
TEE   total energy expenditure
THF   tetrahydrofolate
U   unit
UCB   urine containment bag
UCD   urine collection device
ULLS   unilateral limb suspension
UPA   Urine Processor Assembly
US   United States
UV   ultraviolet
VDR   vitamin D receptor
WHO   World Health Organization
y   year
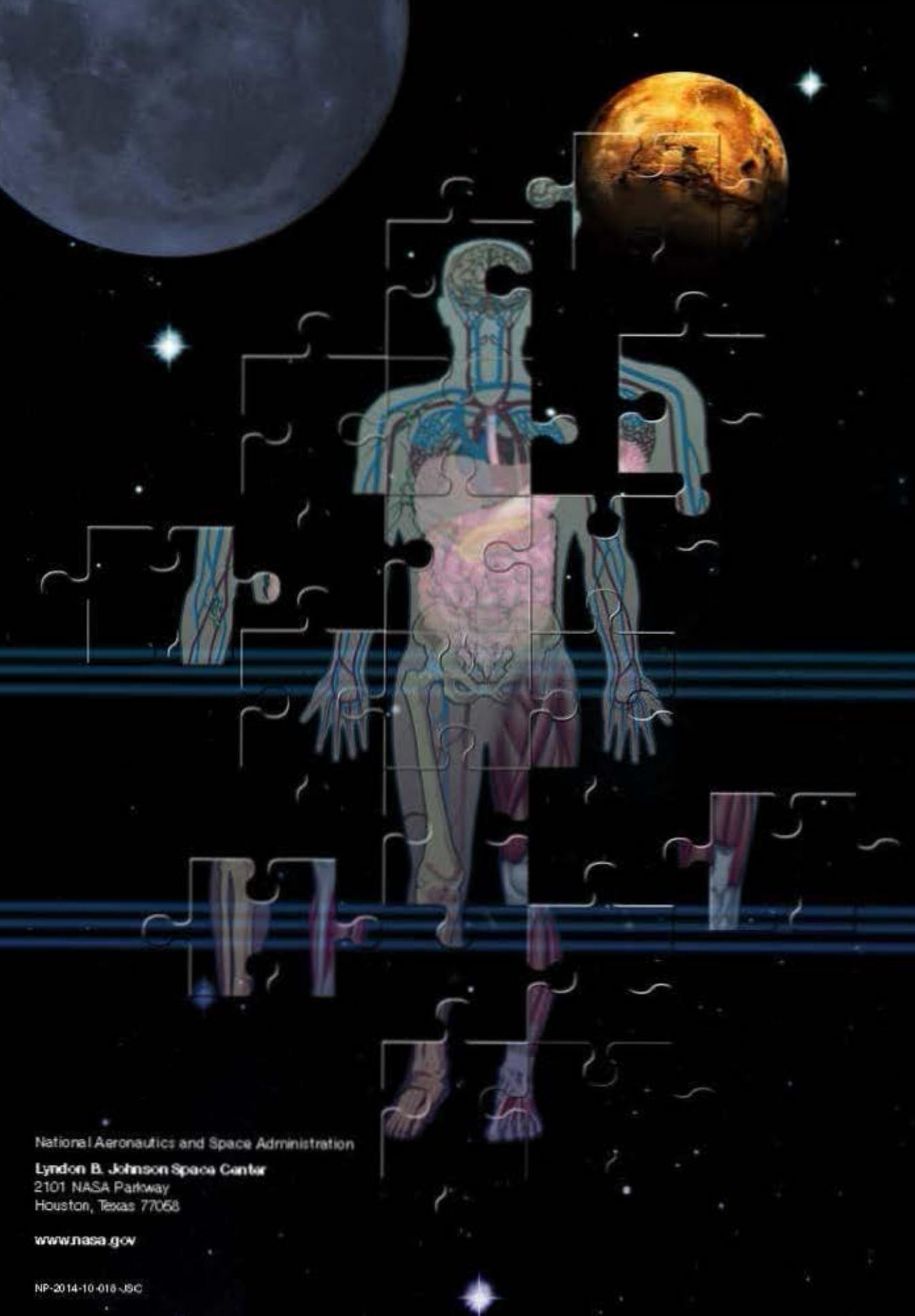
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