

# **Evidence Report:**

## **Risk Factor of Inadequate Nutrition**

### **Human Research Program**

### **Human Health Countermeasures Element**

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## **I. PRD Risk Title: Risk Factor of Inadequate Nutrition**

**Description:** It is critical that crewmembers be adequately nourished before, during, and after space missions (1, 2). Required research areas within this risk include validation of the correct nutritional needs, assessment of the stability of nutrients during long-duration flight, correct packaging and preservation techniques, effects of countermeasures on nutrition, and use of nutrients as countermeasures.

**Risk Statement:** Given that adequate nutrition is a key factor in all physiological functions, that spaceflight has been shown to alter many physiological functions in humans, and that countermeasures for individual systems may alter nutritional status, there is a possibility that inadequate and/or suboptimal nutrition will compromise crew health, including endurance, muscle mass and strength, immune system function, bone mass and strength, cardiovascular performance, gastrointestinal function, endocrine function, ocular health, psychological health, behavior and performance, and ability to mitigate oxidative damage.

## **II. Executive Summary**

The importance of nutrition in exploration has been documented repeatedly throughout history, where, for example, in the period between Columbus' voyage in 1492 and the invention of the steam engine, scurvy resulted in more sailor deaths than all other causes of death combined (3). Because nutrients are required for the structure and function of every cell and every system in the body, defining the nutrient requirements for spaceflight and ensuring provision and intake of those nutrients are primary issues for crew health and mission success. Unique aspects of nutrition during space travel include the overarching physiological adaptation to weightlessness, psychological adaptation to extreme and remote environments, and the ability of nutrition and nutrients to serve as countermeasures to ameliorate the negative effects of spaceflight on the human body. Key areas of clinical concern for long-duration spaceflight include loss of body mass (general inadequate food intake), bone and muscle loss, cardiovascular and immune system decrements, increased radiation exposure and oxidative stress, vision and ophthalmic changes, behavior and performance, nutrient supply during extravehicular activity, and general depletion of body nutrient stores because of inadequate food supply, inadequate food intake, increased metabolism, and/or irreversible loss of nutrients. These topics are reviewed herein, based on the current gap structure.

### **III. Introduction**

This document is based on many sources of information, including the nutritional requirements defined in 1991 for Space Station Freedom missions (2, 4), the updated set of requirements developed in 1995 (in collaboration with Russian partners) for Mir flights (5), the available published data from short-duration Space Shuttle flights, and longer Skylab, Mir, and International Space Station (ISS) flights.

The initial Human Research Program (HRP) Nutrition Evidence Book was completed in 2008, and a modified version of that document was published in 2009 (2) (available for free download at <http://go.nasa.gov/QS1KW1>). What follows is an update completed in 2014, which has been reorganized to reflect updates and changes to the approved Nutrition Discipline Gaps. **For full disclosure – Almost all of the text of this document is directly taken from these two original publications (2, 6). Any thought that the document herein is “original” writing should be abandoned at this point.** While the 2009 book (2) took more of a nutrient-based approach, the 2014 book (6) took a systems-based approach. Both approaches are included herein, as the knowledge gaps also follow these two paths.

#### **IV. Gap Structure**

The Nutrition Discipline is part of the Human Health Countermeasures Element of the NASA Human Research Program (HRP). The Nutrition Discipline currently manages several Gaps relating to nutrition within the Risk Factor of Inadequate Nutrition. As of this writing in late 2014, the approved Gap structure is as follows:

N3.1: Determine the macronutrient requirements for spaceflight.

N3.2: Determine the micronutrient requirements for spaceflight.

N3.3: We need to determine changes in nutritional status due to spaceflight.

N4: Does mission architecture and/or available countermeasures impact nutritional status of crewmembers during spaceflight?

N6: What impact does the spaceflight environment have on oxidative damage?

N7.1: We need to identify the most important nutritional factors for musculoskeletal health.

N7.2: We need to identify the most important nutritional factors for cardiovascular health.

N7.3: We need to identify the most important nutritional factors for ophthalmic health.

N7.4: We need to identify the most important nutritional factors for behavior and performance.

N7.5: We need to identify the most important nutritional factors for immune health.

N13: Can renal stone risk be decreased using nutritional countermeasures?

N15: We need to identify the most important nutritional factors for oxidative damage during spaceflight.

The Nutrition Gaps, Integrated Research Plan Rev F, and Human Research Roadmap structure are focused to define progress to gap closure and risk mitigation. Gaps N3.1, 3.2, 3.3, and 4 are the core gaps that are aimed at directly mitigating the risk factor of nutrition by addressing nutritional status and requirements, and the impact of countermeasures on nutrition. The remaining gaps are focused on nutrition as a major risk factor for other HRP Risks or physiological systems such as Oxidative stress, Muscle/Bone, Immune, Cardiovascular, Visual Impairment/Intracranial Pressure, and Behavior and Performance. A Gap addressing issues of drug and nutrient interaction needs to be added to the Integrated Research Plan with the next update.

**V. Gap N3.1: Determine the macronutrient requirements for spaceflight.**

**A. Food and Energy**

**1. Background**

Ensuring that the spacecraft food systems provide palatable, safe, and nutritious foods is obviously critical for any space mission. The longer space station missions have included semi-closed food systems, with periodic resupply and transient exposure to unique and fresh foods (7-10). Exploration missions will have a more closed food system, with the potential for supplementation with food sources grown in situ (7, 8, 10).

From the early days of the U.S. space program (11-16), development of foods for spaceflight has proven a significant challenge, yet the design criteria have changed little since then: minimal crumbling, ease of preparation and consumption in microgravity, minimal trash volume, and high palatability. With one exception, the food systems used in every space program to date have been entirely shelf-stable, and they are composed primarily of rehydratable or thermostabilized food items (7, 10). Although these foods are known to have lower hedonistic value (palatability) than fresh or frozen foods, ground-based studies have clearly shown that the Shuttle food system can adequately support most nutritional requirements (17). Skylab is the only U.S. program that has included frozen foods (7, 10).

Energy itself is not readily stored in the body, but the substrates for energy are. Energy in the form of heat is obtained by oxidizing carbohydrates, fats, proteins, and alcohol; it is also known as the heat of combustion. Fat provides the most energy of these sources, at about 9 kcal/gram. Carbohydrates and proteins provide about 4 kcal/gram, and alcohol provides about 7 kcal/gram. Because the body can adapt to different energy sources, large variations in macronutrient intake are generally well tolerated. Adipose tissue is the only viable long-term source of stored energy. Carbohydrate stored as glycogen in liver and muscle provides a transient (hours) source of carbohydrate. Protein can be broken down to release amino acids, but this is done at the expense of muscle tissue.

The estimated energy requirements (EER) for space missions are based on total energy expenditure (TEE), as calculated from the 2002 Institute of Medicine Dietary Reference Intake reports (18), using an activity factor of 1.25 (active) along with the individual's age, body mass (kg), and height (m) in the following calculations:

EER for men 19 years and older

$$\text{EER} = 622 - 9.53 \times \text{Age [y]} + 1.25 \times (15.9 \times \text{Mass [kg]} + 539.6 \times \text{Ht [m]})$$

EER for women 19 years and older

$$\text{EER} = 354 - 6.91 \times \text{Age [y]} + 1.25 \times (9.36 \times \text{Mass [kg]} + 726 \times \text{Ht [m]})$$

## **2. Evidence**

### **Energy Expenditure**

Energy expenditure is often hypothesized to be lower during flight than on the ground, because of the presumed relative hypokinesia in space (13). 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 1g (19). This was determined through indirect calorimetry in the space suit. However, Space Shuttle crewmembers did not have any change in energy expenditure during EVA relative to before flight (20).

Studies of total energy expenditure of Shuttle astronauts documented that in-flight energy expenditure was unchanged from preflight levels (21), or in cases where these crews performed intensive exercise during the mission, energy expenditure during flight was higher than before flight (22). For these studies, the doubly-labeled water (water enriched with deuterium and  $^{18}\text{O}$ ) technique was used to determine oxygen consumption (23). 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 1g, 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 spaceflight, resting energy expenditure did not change, but TEE was less during bed rest than before bed rest (24). Because TEE during flight is unchanged (21) or increased (22) 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 spaceflight is the lack of a metabolic response to stress during bed rest (25). 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 spaceflight on energy and fuel metabolism (26).

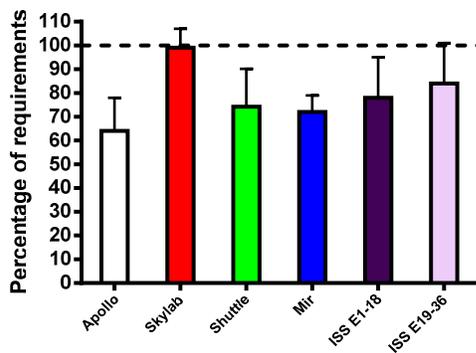
Energy requirements are typically estimated using standard equations, including the World Health Organization (WHO) (27) and dietary reference intake (DRI) equations (18), using a “moderately active” or “active” adjustment for activity level for these 2 equations, respectively. The DRI equation factors in 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 investigators 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 spaceflight, and have occurred on many missions and programs (2, 9, 21, 28-37). From Apollo through the Shuttle program, crewmember dietary intakes during flight have averaged about 70% of predicted requirements (31), with ISS intakes on average about 80% of requirements (Figure 1). There are exceptions to this finding, including the Skylab missions of the early 1970s (38, 39), European flights to the Mir space station (40), and more recently some of the ISS missions (41). 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.

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 (31, 41).



**Figure 1.** In-flight dietary intake of crewmembers in different space programs. Data are expressed as percentage of energy requirements predicted by the World Health Organization (WHO) (27). 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 (7). Figure from (6), adapted from earlier publications (2, 42), with additional published data included (1, 31, 41).

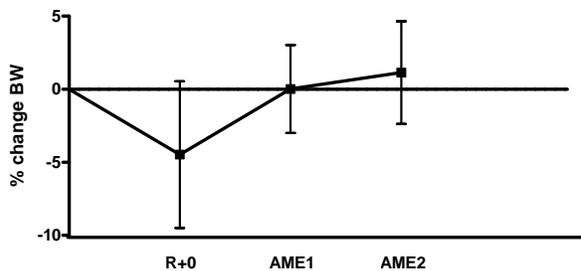
In cases where energy intakes do not meet requirements, absent definitive causes, many potential explanations have been proposed (13, 33, 43). Anecdotal reports of appetite vary significantly, as indicated in a Russian study in which 40% of Mir crewmembers reported decreased appetite, 40% reported no change, and 20% reported increased appetite (44).

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 (45-47). One hypothesis to explain these changes 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 (45). Experimental research has not been able to clearly document changes in taste or olfaction during spaceflight or head-down-tilt bed rest (45, 48, 49), 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  $-6^\circ$  head-down bed rest period, subjects reported decreased appetite and lack of taste early in the bed rest phase (49, 50). By day 13 of the bed rest phase, for all tastes (sweet, salt, acidic, bitter), the threshold for taste sensitivity had increased. In contrast, in the 1990s no changes in odor or taste perception were found after 14 days of head-down bed rest (51), 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  $-6^\circ$  head-down bed rest, mouth-to-cecum transit time was significantly longer than it was during ambulatory control periods (52). 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 spaceflight, in humans and in animal models, have previously been reviewed (53). A common cause of reduced dietary intake during the first days of a mission (54) is space motion sickness (47, 54-57). 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 (43).

Anecdotal reports from long-duration crewmembers indicate that a rebound body mass gain occurred after flights on which on-orbit loss of body mass was significant, but in general, the data do not support this finding (Figure 2) (2).

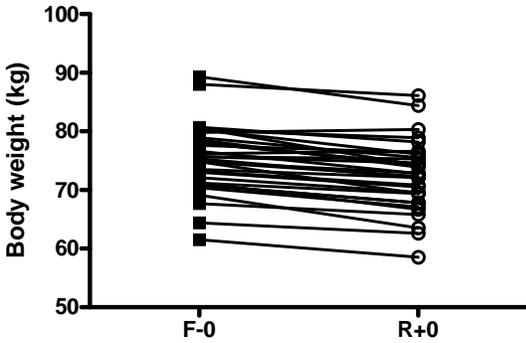


**Figure 2.** Postflight body weight (BW) in Mir and ISS crewmembers (N=20). Data are expressed as the mean  $\pm$  SD of the percentage change from preflight body weight. R+0 = landing day, and AME1 and AME2 = first and second annual medical exams after return from the mission, respectively. (2)

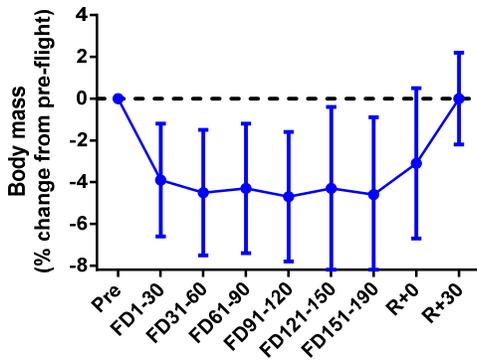
### **Implications for Inadequate Energy Intake**

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 spaceflight, although some crewmembers have been able to maintain body mass (31, 41, 58). Indeed, all crewmembers on Gemini, Apollo (Figure 3), Skylab (Figure 4), and Apollo-Soyuz Test Project missions lost body mass (59); thus, ingestion of the prescribed energy intake on the U.S. Skylab missions did not ensure maintenance of body mass (38). In-flight and postflight losses of body mass are shown in Figure 4 and Figure 5. Documented weight losses have occurred on short- and long-duration flights in both the U.S. and Russian space programs (14, 33, 60-62). In one study

of 13 male Shuttle crewmembers, body mass losses ranged from 0 to 3.9 kg (21). Body mass loss has been observed to reach 10% to 15% of preflight body mass (63). Crewmembers on ISS (Figure 4 and Figure 5) have shown similar patterns of mass loss during and after flight (60).

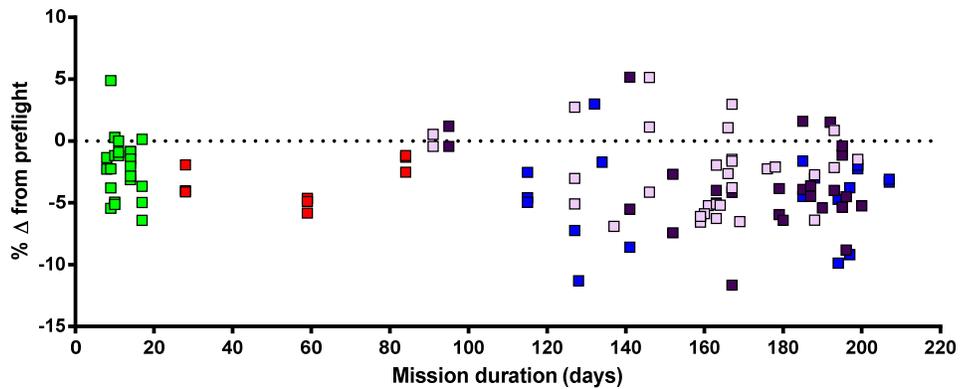


**Figure 3.** Body weight in Apollo crewmembers (Apollo 7 through 17) before (F-0) and after (R+0) flight (64).



**Figure 4.** In-flight body mass measurement data from 55 ISS crewmembers. Data are expressed as percentage change from preflight values. Figure and data adapted from Zwart et al (60).

**Figure 5.** Body mass on landing day expressed as percentage change from preflight. Each symbol represents a crewmember from a mission on the Space Shuttle (green, N=25), Skylab (red, N=9), Mir (blue squares, N=19), ISS Expeditions 1-18 (dark purple, N=26), or Expeditions 19-36 (light purple, N=31). Some durations have been adjusted slightly to ensure anonymity. Data updated from initial publications (2, 6, 10).



## Risk Factor of Inadequate Nutrition

Data relating reduced dietary intake to loss of body mass were collected from two ground-based studies, not related to spaceflight, in which subjects were semi-starved. In the first study (65), 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 (66).

Data from Apollo missions clearly document the relationship between energy intake and weight loss (Figure 6). The mechanism for this energy-weight/mass connection has been hypothesized to involve multiple functions of many endocrine factors, including insulin, leptin, and growth hormone (67).

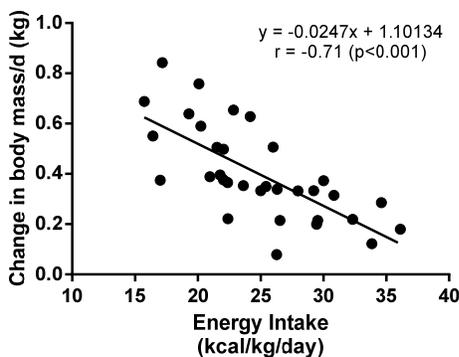


Figure 6. Relationship between energy intake (kcal/kg body mass/d) and weight loss (change in body mass/d, kg) during Apollo missions. N=33. Data are courtesy of William Carpentier, as originally presented in (6).

Only about 1% of the loss of body mass can be explained by loss of body water (28); most of the observed loss of body mass is accounted for by loss of muscle and fat tissue (20, 68). 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 (Figure 7), but also with decreased protein synthesis (69) (during spaceflight), increased protein catabolism (70) (during bed rest), and subsequent loss of lean tissue mass.

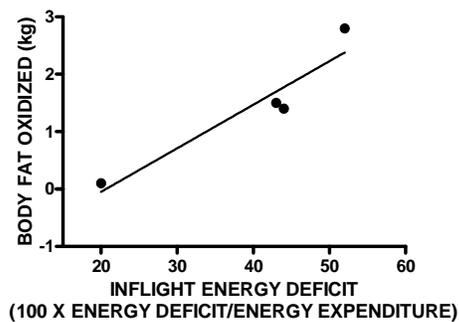


Figure 7. In-flight oxidation of body fat related to in-flight energy deficit. Adapted from (22).

Besides the obvious concerns about body mass loss and dehydration (71), 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 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. Results for body mass and protein metabolism, calcium loss, and LBNP “survival” are shown in Figure 8, Figure 9, Figure 10, and Figure 11. These clearly document the negative effects of undernutrition, exacerbating the negative effects of bed rest. These data document the fact that undernutrition exacerbates the negative effects of bed rest on human physiology (2, 6, 70).

Figure 8. Body weight changes in a crossover-design bed rest study to evaluate the hypocaloric impact of nutrition on integrated physiology. Data are expressed as the change from the group average body weight before bed rest (6).

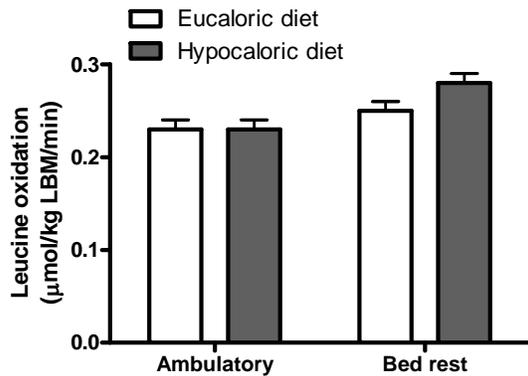
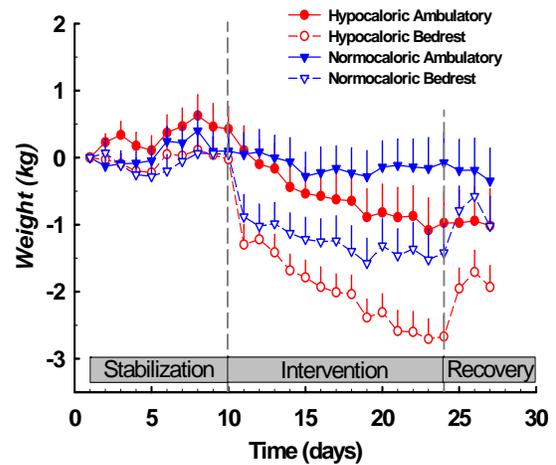


Figure 9. Leucine oxidation (an index of net protein catabolism) in a crossover-design bed rest study to evaluate the impact of hypocaloric nutrition on integrated physiology. There was a significant ( $P=0.04$ ) interaction between bed rest and diet (adapted from (70)).

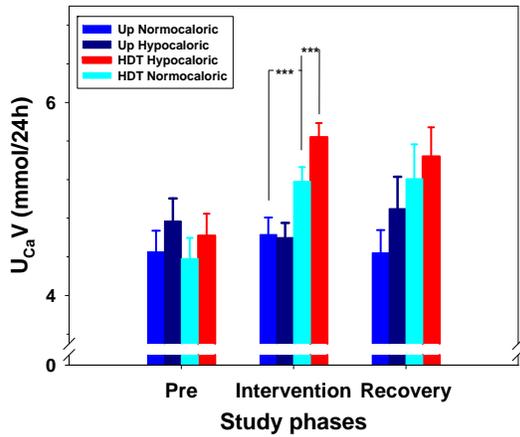


Figure 10. Urinary calcium in a crossover-design bed rest study to evaluate the impact of hypocaloric nutrition on integrated physiology. Data are expressed as mmol Ca excreted per 24 hours before, during, and after bed rest. From inset legend: Up, ambulatory phase; HDT, head-down-tilt bed rest phase.

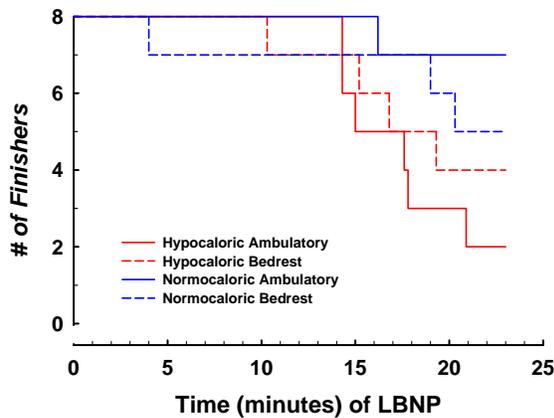


Figure 11. Lower-body negative pressure (LBNP) “survival” in a crossover-design bed rest study to evaluate the impact of hypocaloric nutrition on integrated physiology. Data are expressed as the number of subjects who were able to tolerate different amounts (minutes) of LBNP (72).

### 3. Risk

It is imperative that adequate resources be provided to support food consumption. A reliable food system must include a variety of palatable foods and the means to process them (such as rehydration, heating, and cooling). Time (for meal preparation, consumption, and cleanup) is another limited resource that often hinders dietary intake during spaceflight.

The availability 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 energy leads to wasting and ultimately tissue breakdown, or even death. An excess of energy may lead to excess body mass if metabolic rate does not increase. The loss of lean body mass during spaceflight is significant and is associated with increased proteolysis and catabolism related to metabolic stress (73).

The small amount of starvation data available suggest that for every 500 kcal consumed per day, about 1% of body mass can be conserved every 12 days. It would not be acceptable, however, to use these numbers for a long-term (>21 days) prediction of body mass loss or conserved body mass loss because after 21 days of starvation, the basal metabolic rate of the

body decreases (65, 74). This can be and has been accounted for using a mathematical model to predict body mass loss given changes in the basal metabolic rate (74), with results estimating that survival on 1000 kcal/d could exceed 3 years (compared with only 6 months without accounting for a decreased metabolic rate).

It is difficult to predict the impact of suboptimal (or lack of) energy intake in otherwise healthy individuals. One issue is that the energy equivalent of the mass lost changes with time, as different body fuels are used at different times during semi-starvation (65, 74). With partial rations available (1000 calories per day), 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. Greater restrictions in energy availability would be expected to yield survivability ranging 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 (75, 76).

Other possible effects of long-term low calorie intake include decreased motor and cognitive function, both 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 (77). During total fasting, degradation of coordination, speed, and cognitive function would be evident within the first 2 weeks (77).

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).

It is speculated that a crew could survive on a spacecraft or planetary base for 40-60 days without food. With limited rations (1000 calories/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 body mass loss 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, virtually all spaceflight data collected on Shuttle, Mir, and ISS missions are confounded by inadequate dietary intake. Investigators who have studied bone and muscle, cardiovascular function, immune response, and other systems during spaceflight cannot say to what degree undernutrition affected their findings.

#### **4. Gaps**

Studies of energy expenditure have been conducted only on short-duration (Shuttle) flights (21, 22). Whether the same trends continue on longer flights is not known (an ESA-sponsored study of energy expenditure on ISS missions is currently being conducted on ISS). The health implications of this phenomenon need to be determined, and ways to prevent both in-flight body mass loss and postflight body mass gain need to be evaluated.

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 spaceflight (41). 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 (41). Additional details are provided in the Bone section.

Although energy requirements are currently estimated using published equations (see above), valuable and more accurate information would be provided by actually determining resting metabolic rate in astronauts before (if not during) flight. The recent Pro K study (see Bone section) included provision of controlled diets based on estimated energy requirements. While these provisions were adequate for some crews, for others they seemed to provide excess calories, and for others insufficient calories. These differences are likely to be generally due to variability in individuals; again, this could be determined through preflight indirect calorimetry. Such measurements are included in the Integrated Nutrition experiment, but as of this writing (October 2014) there is debate as to whether this experiment will be manifested by the Human Research Program.

With respect to the provision of energy in bed rest studies, the issues of whether and how to account for energy intake in this ground analog are separate. At least two approaches exist to controlling body mass and composition while studying human adaptation to bed rest: either maintaining body mass (as is typically done in the U.S.) 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 effectively has not been possible (to date), given the difficulties in measuring fat mass and adapting intake in a timely manner. In one case, there were significant differences in weight loss between groups, and even between subjects, which confounds interpretation of the findings (78-81). In view of the difference between these two approaches, it has been proposed that a bed rest study of energy expenditure is required to determine the impact of this difference. This yielded nutrition knowledge gap 11 (identified by the Small Assessment Team in 2006) (82), “What model of bed rest energy expenditure is best?”

## **B. Protein**

### **1. Background**

As the major structural component of all cells in the body, protein includes molecules that perform many essential physiological functions, serving as enzymes, hormones, transport carriers, and other important molecules. The total energy contribution of protein to the average diet is about 15%. The nitrogen in their amino-acid building blocks makes protein, along with nucleic acids, one of the major nitrogen-containing macromolecules. The type of protein, such as animal or vegetable protein, that is incorporated into the diet may be an important factor to consider in determining protein requirements.

Protein is one of the most critical 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 33-50% of total body protein is lost, death results (83). Total body mass loss in excess of 40-50% of initial body mass is not compatible with life (77, 84). 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 (75, 76).

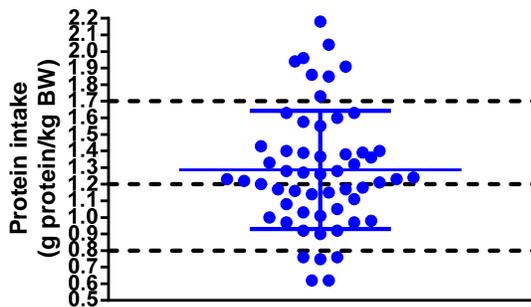
### **2. Evidence**

#### **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 (85, 86). 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 (87, 88).

Actual intakes of protein during spaceflight typically exceed these recommendations, as shown in Appendix A and (2). 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 (9). On ISS missions, on average, protein intake is more than adequate (Figure 12).

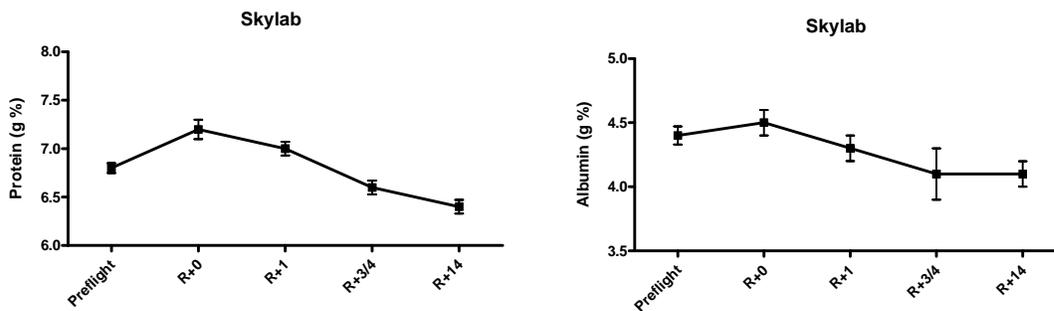
Some data suggest that during the recovery period after short-duration Shuttle flights, protein is a limiting nutrient, and that competition for substrate to replenish plasma proteins and muscle mass strains the system (89). This has not been tested experimentally, but it is clear that good nutrition is required for rapid return to optimal health.



**Figure 12.** Protein intake during spaceflight 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 spaceflight (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 (90). The blue lines represent the mean  $\pm$  SD. Figure from (6).

### Protein Status and Nitrogen Balance

Basic clinical testing includes determinations of circulating proteins. Blood concentrations of total protein and albumin were elevated at landing after Skylab missions (Figure 13). Urinary albumin has been shown to be reduced during spaceflight and bed rest (91-93). 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 (39, 94) despite losses of leg volume (38, 95). Nitrogen balance has also been shown to be negative during Shuttle flights (96)



**Figure 13.** Plasma total protein and albumin in Skylab crewmembers before flight and after landing (R+0, landing day) (39).

### 3. Risk

The risks associated with protein are twofold: deficiency and excess. Deficiency of protein leads to muscle loss, weakness, wasting, tissue breakdown, inability to perform one's job (including egress from the spacecraft), and ultimately death. Excess protein exacerbates hypercalciuria and the risk of renal stone formation, and is detrimental to bone. Furthermore, specific amino acids may additionally increase these risks. This is reviewed in much more detail

under Gap N7.1, “We need to identify the most important nutritional factors for musculoskeletal health.”

#### **4. Gaps**

Research continues on the effects of amino acid supplementation as a means to mitigate muscle loss. This research needs to continue to refine the details (such as dose and timing) and assess the viability of this countermeasure.

Further research is also required to better understand the effects of protein source (animal vs. vegetable, and the effect of sulfur amino acid content) on bone loss and renal stone risk. This has been advocated in context of spaceflight (97-99), and general health (100, 101).

## C. Carbohydrate

### 1. Background

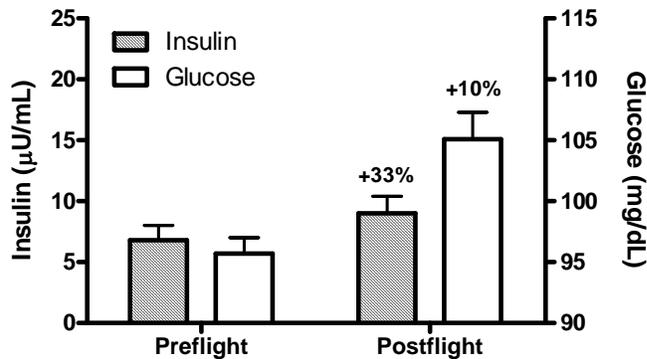
Carbohydrates play an important role in the body because they supply the primary source of energy as well as a readily available source. This energy is oxidized and used by various organs and cells in the body, particularly the brain and red blood cells, which depend solely on carbohydrate for energy. The human body stores about 150 to 500 g of carbohydrate as glycogen, in the liver and skeletal muscle (102). 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 (103). Liver stores of glycogen are depleted after 12 to 18 hours of fasting (102). 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.

### 2. Evidence

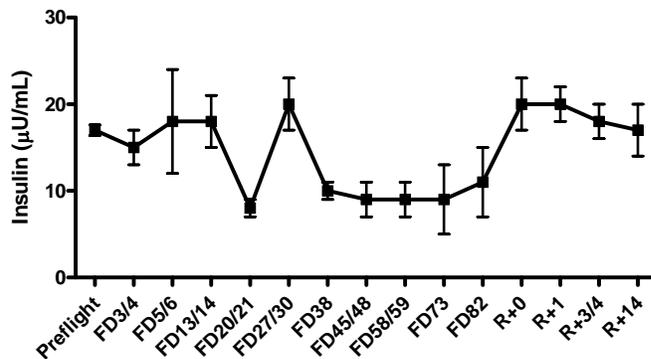
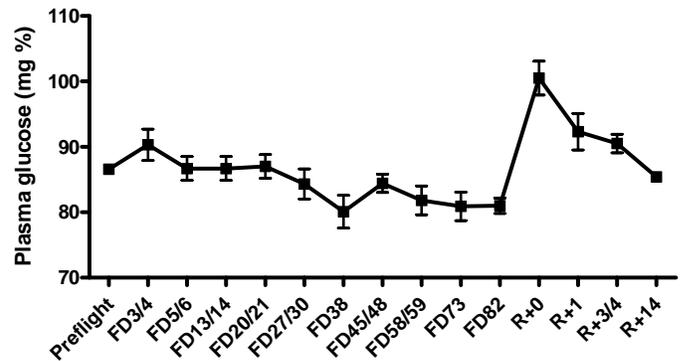
Early studies documented both increased insulin and glucose at landing in Apollo (Figure 14) and Skylab (

Figure 15, Figure 16) flights.



**Figure 14.** Plasma insulin (N=22) and glucose (N=33) in Apollo crewmembers before and after flight (104).

**Figure 15.** Plasma glucose in Skylab crewmembers before, during, and after flight (39).



**Figure 16.** Plasma insulin in Skylab crewmembers before, during, and after flight (39).

On the Shuttle, studies by German investigators showed no impact of 7 days of flight on glucose tolerance tests (105). Additionally, 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 (106, 107). Insulin resistance (lack of sensitivity to insulin) has been found to result from simulated weightlessness (bed rest) (108-111). Using C-peptide excretion as a proxy, Stein et al found evidence of insulin resistance during actual and simulated spaceflight (112). Heer et al documented that after 3 weeks of bed rest, glucose tolerance was altered for more than 4 days after reambulation (113). 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 spaceflight may have consequences for the crew's productivity and impede their ability to respond in emergency situations (114). Deficiency of carbohydrate leads to ketosis. A ketotic state would likely impair performance of crewmembers, as seen in studies conducted by the military (77), as well as increase renal stone risk secondary to reduced urinary pH (115-117). 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 spaceflight on carbohydrate metabolism. Observations from spaceflight as well as ground-based bed rest studies show subtle changes in insulin secretion, insulin resistance, and glucose intolerance (109, 110, 118, 119). 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.

### **Requirements**

The current documented requirement for carbohydrate intake during spaceflight is 50% to 55% of the total daily energy intake. In the U.S., acceptable daily intake of dietary carbohydrate is defined as intake between 55% and 75% of the total dietary energy (120). A minimum intake of 140 g/d is required to maintain the needs of organs that require carbohydrate for energy production (121). For reference, the daily carbohydrate requirement for male and female astronauts was originally defined in 1991 for missions of 30-120 days as 50% of the total energy intake (4) and in 1995 for missions up to 360 days as 50% to 55% of the total energy intake (5), with a caveat that most of the carbohydrate should be provided as complex carbohydrates and less than 10% of the total carbohydrate should be provided as simple sugars.

Macronutrient intake by space crews tends to reflect a relatively high protein and carbohydrate intake (close to 60% of calories) (2, 6, 43). Some researchers have speculated that this may represent a shift in macronutrient preferences, but it may also simply be related to the high sugar content of many of the available beverages in the U.S. food supply.

### **3. Risk**

Suboptimal carbohydrate intake before and during spaceflight may have consequences for the crew's productivity and impede their ability to respond in emergency situations (114).

Deficiency of carbohydrate leads to ketosis. A ketotic state would likely impair performance of crewmembers, as seen in studies conducted by the military (77), as well as increase renal stone risk secondary to reduced pH (115-117). Other aspects of the mission would also be at risk (for example, the life-support systems may not be able to remove the ketones from the air). Toxicity of carbohydrate has not been well studied and would likely be an issue only with regard to displacement of other nutrients (protein and fat).

### **4. Gaps**

Few data are currently available to assess the impact of spaceflight on carbohydrate metabolism. Observations from spaceflight as well as ground-based bed rest studies show subtle changes in insulin secretion, insulin resistance, and glucose intolerance (109, 110, 118, 119). Even subtle changes in such important metabolic processes make it critically important to consider the possibilities of altered carbohydrate and insulin metabolism for exploration missions.

## **D. Dietary Fiber**

### **1. Background**

Dietary fiber consists of non-digestible food components that are typically carbohydrate and plant based. Non-starch polysaccharides, including cellulose, gums, pectins, mixed-linkage  $\beta$ -glucans, and hemicelluloses, are the major components of dietary fiber. Lignan is also included even though it is a non-carbohydrate component.

A role for dietary fiber has been implicated in decreases in plasma cholesterol, modification of the response of blood glucose to food (the glycemic response), improvements in large bowel function, and decreases in the bioavailability of some nutrients. Epidemiological evidence also points to relationships between diets high in fiber and decreased incidence of cardiovascular disease and bowel cancer (122).

### **2. Evidence**

Changes have been described in gastrointestinal function and gut transit time during spaceflight. Adequate dietary fiber will be essential to maintaining gastrointestinal function and decrease the incidence of constipation because mouth-to-cecum transit times are slower on orbit (114).

The current documented requirement for dietary fiber intake in spaceflight is 10-14 g/1000 kcal. In the U.S., acceptable daily intake requirements for dietary fiber are provided (18) for individuals aged 19-50 years (men 38 g/d and women 25 g/d) and for individuals aged 51-70 years (men 30 g/d and women 21 g/d).

For reference, the daily total fiber requirements for male and female astronauts were defined in 1991 for missions of 30-120 days as 10-15 g, in soluble and insoluble forms (4), and in 1995 for missions up to 360 days as 10-25 g, in soluble and insoluble forms (5).

### **3. Risk**

Inadequate dietary fiber, in combination with low fluid intake, may lead to constipation. There is an open question about the effectiveness of vitamin K synthesis by colonic microflora (and its availability) during spaceflight (see vitamin K section).

### **4. Gaps**

Several studies have shown that specific dietary fatty acids and types of dietary fiber can reduce animals' risk of developing radiation-induced cancer (123, 124). Further research is warranted to investigate the potential protective effects of fiber on radiation-induced cancer risk in humans exposed to high-linear energy transfer radiation during spaceflight.

## E. Fat

### 1. Background

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. Body stores of fat are located mainly in adipose tissue as triacylglycerols. Adipose tissue is dispersed throughout the human body, with its distribution patterns differing between sexes.

According to case studies, people following fat-free diets can exhibit symptoms of essential fatty acid deficiencies after only 1 month. In one study, an infant consuming fat-free total parenteral nutrition for 3 months developed skin lesions and had polyunsaturated fatty acid levels of less than 10% of control values (125). In another study, an adult consumed fat-free total parenteral nutrition for 7 months and developed severe dermatitis by the end of the first month. Omega-3 (n-3) fatty acids constituted 0.01% of the fatty acids of this person's plasma phospholipids, which means that the patient was almost completely depleted of n-3 fatty acids (126).

### 2. Evidence

While few (if any) studies have been conducted to investigate dietary fat, plasma lipid levels, and related factors during spaceflight, numerous data exist from routine medical exams conducted before and after flight, along with annual medical examinations (Figure 17, Figure 18, Figure 19).

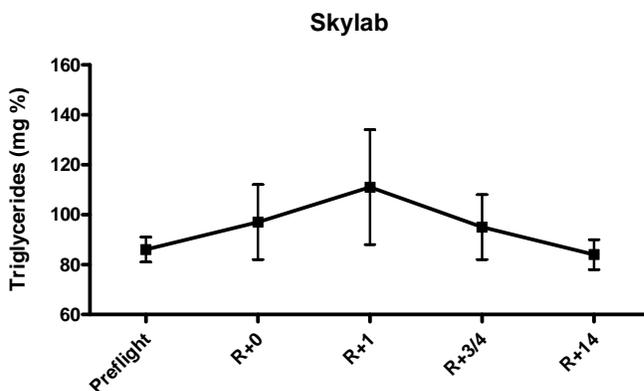
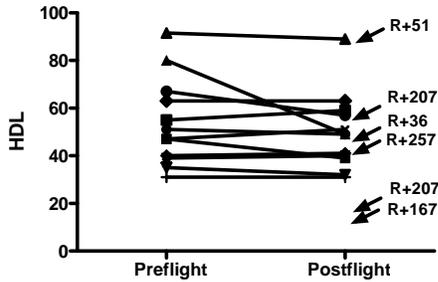
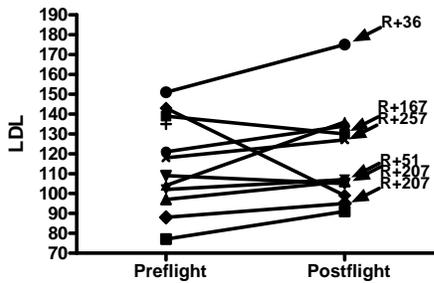


Figure 17. Plasma triglycerides in Skylab crewmembers before and after flight (39).

Risk Factor of Inadequate Nutrition

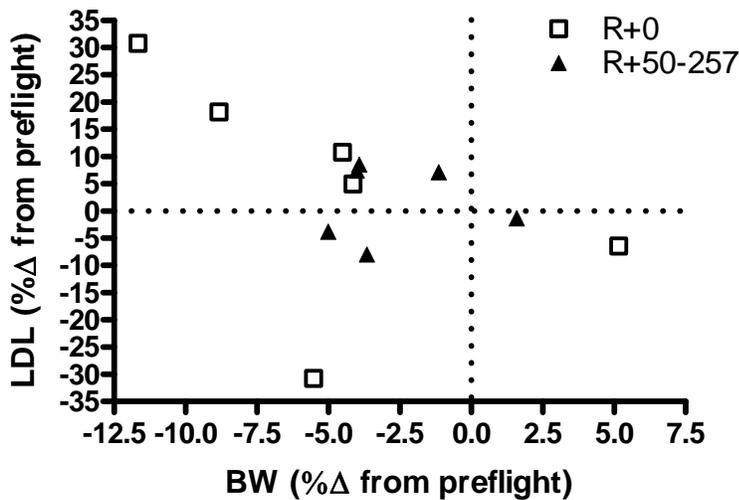


**Figure 18.** Serum high-density lipoproteins (HDLs) in ISS crewmembers before and after flight. Unless otherwise noted, data are from R+0/1. Because HDLs are not routinely measured at landing, some data were available only at the next medical exam. In these cases, the date is denoted on the chart as R+X days (2).



**Figure 19.** Serum low-density lipoproteins (LDLs) in ISS crewmembers before and after flight. Because LSLs are not routinely measured at landing, some data were available only at the next medical exam. In these cases, the date is denoted on the chart as R+X days (2).

Contrary to the typical lipoprotein response to weight loss, LDL concentrations tended to increase in long-duration crewmembers who lost weight during the flight. This relationship seems to return to normal by the subsequent medical exam (based on available data, 50-257 days after landing) (Figure 20) (2, 6).

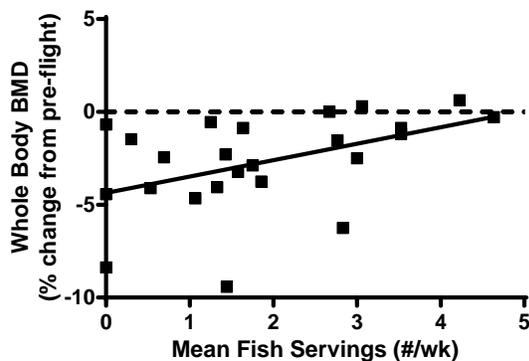


**Figure 20.** Relationship between the loss of body mass post flight and the change in serum LDLs in ISS crewmembers. Because LDLs are not routinely measured at landing, some data were available only at the next medical exam, which ranged from 50 to 257 days after landing.

Alterations in fuel homeostasis and regulatory hormones have been noted in spaceflight and ground-based studies. Bed rest studies have documented alterations in fuel homeostasis (127), including sex differences (111). Specifically, lipogenesis increased during bed rest, to a greater extent in women than in men. Additionally, men had increased carbohydrate oxidation (111). Other studies have shown inflammatory changes during bed rest, along with insulin resistance, leading to increased body fat and altered fatty acid metabolism (128). Given these data, and the insulin, leptin, and other endocrine changes noted in bed rest and spaceflight (25, 129, 130), 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 spaceflight radiation effects (123, 124). Not only do omega-3 fatty acids (in combination with pectin) show promise in alleviating cancer risk (123, 124, 131-133), 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 (134-143), indicating the likelihood is high that eicosapentaenoic acid will have the same beneficial effects on muscle atrophy during spaceflight or in ground-based analogs including bed rest.

Omega-3 fatty acids also have been shown to protect bone, in the general population (144-146) as well as in spaceflight analog studies, including bed rest and cell culture (147). Although omega-3 fatty acids have not been studied in a controlled fashion during actual spaceflight, a positive correlation was found between fish intake and bone loss in astronauts (147). That is, those who ate more fish lost less bone (Figure 21). These data provide additional evidence of the potential importance of fish oils as a countermeasure for muscle, bone, and radiation risks of spaceflight. Studies showing positive effects of omega-3 fatty acids typically look at intake of fish or other food sources of these nutrients (148-150). Studies of fish oil supplements added to typical diets often fail to document any benefit (151-153), thus highlighting the need for dietary modification, and not simply supplementation.



**Figure 21.** Relationship between fish intake during flight and whole-body bone mineral density loss after flight. Figure adapted from (147).

## **Requirements**

The current documented spaceflight requirement is for dietary intake of fat to constitute 25% to 35% of the total daily energy intake (97). Dietary intake of n-6 and n-3 fatty acids should be 14 g/d and 1.1-1.6 g/d, respectively. Consumption of saturated fat should be <7% of calories, consumption of trans fatty acids <1% of total calories, and consumption of cholesterol <300 mg/d. In the U.S., no recommended dietary allowance or adequate intake level has been set for total fat because data are insufficient to determine the level of dietary fat that may put one at risk for inadequacy or may contribute to the prevention of chronic disease (18). The acceptable distribution range for fat intake is 20% to 35% of the total energy intake (18). For reference, the daily total fat requirement for male and female astronauts was defined in 1991 for missions of 30-120 days to be 30% to 35% of the total energy intake (4) and in 1995 for missions up to 360 days to be 30% to 35% of the total energy intake (5).

### **3. Risk**

Deficiency of fat leads to essential fatty acid deficiency and ultimately death. Toxic levels of fat lead to high serum cholesterol, obesity, atherosclerotic plaques, and, ultimately, coronary heart disease, or even death.

### **4. Gaps**

The role of n-3 fatty acids in many systems has been investigated to one degree or another. Studies of n-3 fatty acids and cancer prevention have been investigated in animal models of spaceflight radiation effects (124). Not only do n-3 fatty acids (in combination with pectin) show promise in alleviating cancer risk (123, 124, 131-133), 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 conditions, such as cancer or sepsis (134-140, 143), indicating the likelihood is high that eicosapentaenoic acid will have the same beneficial effects on muscle atrophy during spaceflight or ground-based analogs, including bed rest (98). As mentioned above, fish intake and omega-3 fatty acids are also documented to protect bone in ground research, ground analog research, and preliminary spaceflight analyses. Thus, further research on omega-3 fatty acids, and eicosapentaenoic acid specifically, is warranted.

## **F. Fluid Homeostasis and Renal Stone Risk**

### **1. Background**

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 (154).

Total body water makes up about 50% to 70% of body mass (155). Fluid requirements increase with metabolic rate and heat stress. Death from dehydration can occur within weeks of depriving the body of all water (156).

### **2. Evidence**

Fluid and electrolyte homeostasis is significantly altered during spaceflight, and this has been extensively reviewed (28, 40, 157-164). 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 spaceflight; 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 shift has never been confirmed during actual spaceflight (28, 158, 163, 165-167).

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 (28, 168). 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 (28). The idea that interstitial fluid volume is conserved is supported by rapid decreases in total circulating protein, specifically albumin (28), 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 (28).

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 (28). Plasma volume was partially restored during this period, from the initial ~17% below preflight levels to ~11% below preflight levels (28), and it has been found to remain 10% to 15% below preflight levels even for extended-duration flights (169).

Leach et al (28) and Norsk et al (165) 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 (170). Furthermore, the loss of protein may in part explain why fluid loading alone does not restore circulatory volume (171, 172), 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 (173).

The effect of spaceflight 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 (28, 174, 175), 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 (163, 165, 167, 176, 177).

Diuresis is also typically not observed during flight (68, 157, 158, 165, 167, 176, 178-180), for a number of possible reasons. Operational constraints have made it difficult to document urine volume accurately on the first day of spaceflight. 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 (28). Urine volumes on a week-long flight to Mir were also less than preflight volumes (179). During the first week of the 59- and 84-day Skylab flights (39), 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 (181-184).

As mentioned above, the percentage of body mass represented by total body water is relatively unchanged during flight (28). 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 (28). Thus, by difference, intracellular fluid volume increased during spaceflight. This had been previously hypothesized from ground-based studies (185) and observed in postflight studies of Apollo crewmembers (68). The mechanism for a spaceflight-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 (186-188). Urinary albumin, a marker of kidney function, has been shown to be reduced in both spaceflight (relative to before flight) and bed rest (relative to the ambulatory state) (91-93). However, spaceflight, but not bed rest, results in reduced urine flow rates (167). Taken together, these data suggest that differences in fluid metabolism exist between analog studies and actual spaceflight (163, 165-167, 177, 180, 188). Such differences do not seem to be a simple effect of abnormal renal function, and thus require further investigation (189).

Although no spaceflight-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 is 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 spaceflight, where water is a limited commodity, is extremely low.

Decreased fluid intake during spaceflight may be a consequence of reduced thirst during flight (114), 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).

In the U.S., the recommended total water intake (including the water contained in food, beverages, and drinking water) is 3.7 L/d for men (19 y and older) and 2.7 L/d for women (19 y and older) (190). This is considered “adequate intake.” Since 1991 (4, 5), the spaceflight requirement for fluid has been 1-1.5 mL/kcal, with a minimum intake of 2000 mL/d. Actual fluid intakes on average meet this minimum (>2 L) requirement, but not often enough (2, 191).

Diuresis has been documented in bed rest studies (186), suggesting that differences in fluid metabolism exist between analog studies and actual spaceflight (163, 166, 180).

### **3. Risk**

Although no spaceflight-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 wherever possible.

Deficiency of fluid leads to dehydration and ultimately death. Likewise, an excess of fluid intake leads to water intoxication or even death.

### **4. Gaps**

Decreased fluid intake during spaceflight may be a consequence of reduced thirst during flight (114), but the reason for reduced thirst is unknown.

The studies described above have documented that total body water is unchanged during flight, but there is an apparent shift of fluid from the extracellular to the intracellular compartment. The effect of this 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. This might be responsible for some of the microgravity-induced changes noted in other systems (such as the endocrine, cardiovascular, and immune systems).

## **G. Sodium and Chloride**

### **1. Background**

Sodium is the major cation of extracellular fluid (154). 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 (192). Electrolyte concentrations in the body are essential for proper cardiovascular function and are under renal and hormonal control (193). 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 (194). Exchangeable sodium becomes available by diffusion when plasma sodium levels become low, and in states of edema, the exchangeable sodium stores absorb sodium.

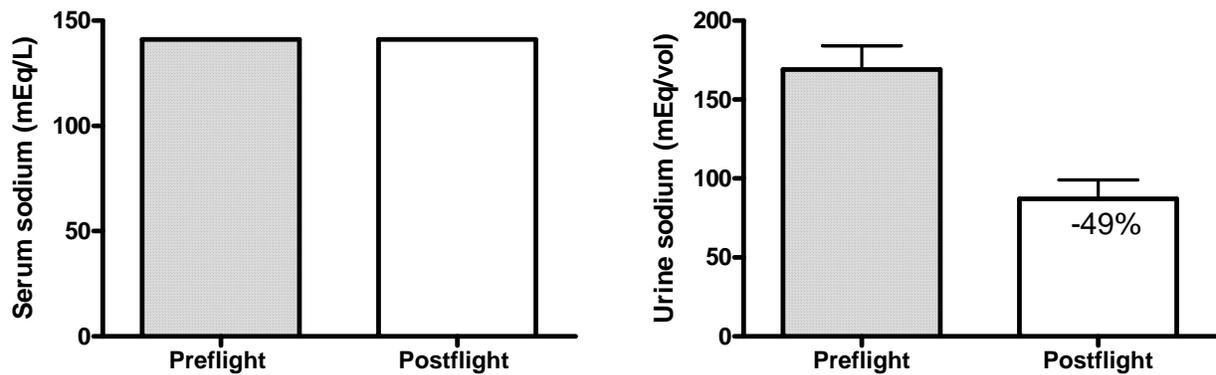
Animal studies show that symptoms of sodium deficiency occur after 3 to 4 weeks of dietary sodium restriction (195). During acute starvation, urinary sodium excretion decreases to less than 0.2 g within 10 days (196), and can be affected by the amount of sweat (197). Plasma sodium levels are maintained fairly well during acute starvation: an initial decrease is followed by a return toward normal values (198). 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  $0.6 \pm 7.3\%$  higher than baseline levels (N=4) (197). Six days of undernutrition resulted in large negative balances of sodium chloride ( $-12.8 \pm 3.6$  g/d), likely related to changes in water balance (197).

### **2. Evidence**

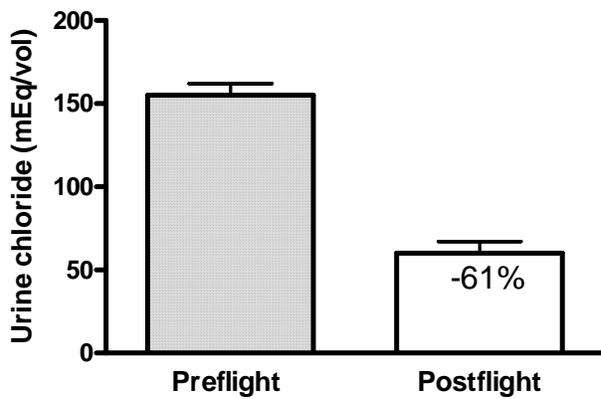
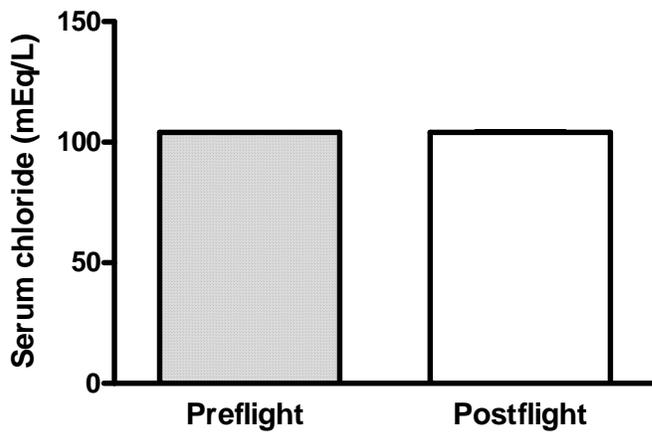
Pre-, in-, and postflight plasma sodium and chloride data are available from Apollo (Figure 22, Figure 23), Skylab (Figure 24, Figure 25), and Shuttle flights (Figure 26), and have been reviewed extensively (2, 9, 158, 165, 166, 199). In-flight sodium intakes during Skylab and Shuttle missions averaged 4 to 5 g, and were similar to the astronauts' preflight intakes (7). 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 (31). 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 spaceflight (200), 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 spaceflight, in a non-osmotic fashion (that is, without a concomitant increase in fluid compartments) (9, 40, 165, 166, 173, 176). These data were confirmed in a series of ground-

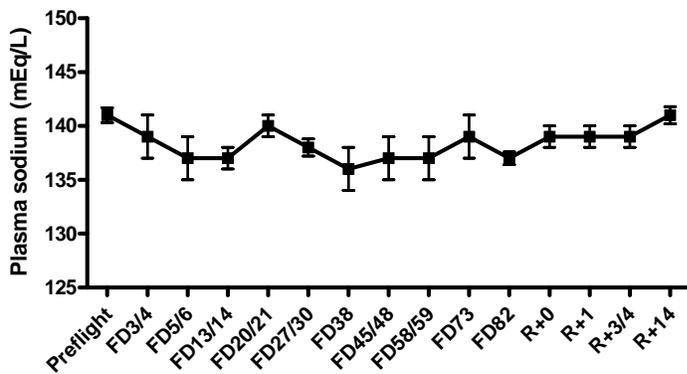
based studies, documenting an increase in messenger RNA (mRNA) expression of some of the enzymes required for glycosaminoglycan syntheses in the skin, the displacement of sodium by hydrogen in the glycosaminoglycans, and a subsequent acidosis (173, 201-206). These findings of increased sodium-proteoglycan binding had already been observed in animal studies by Ivanova in the 1970s (207). 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 (201, 202, 204) in animals and summarized in a mini-review (204), the group 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.



**Figure 22.** Serum (N=33) and urinary (N=30) sodium from Apollo crewmembers. Numbers in bars represent the percentage change from preflight values. Adapted from (104).

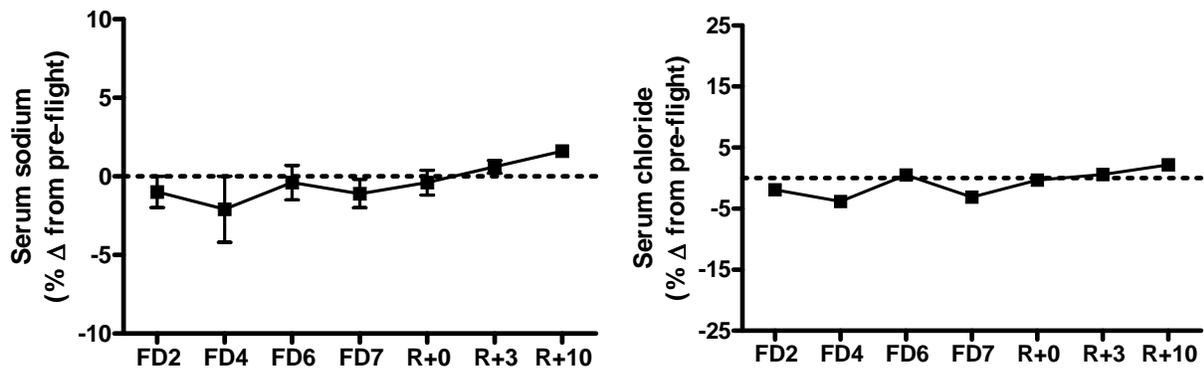
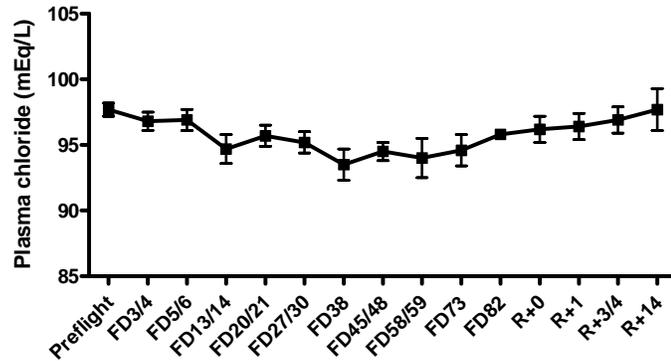


**Figure 23.** Serum (N=33) and urinary (N=30) chloride from Apollo crewmembers. Numbers in bars represent the percentage change from preflight values. Adapted from (104).



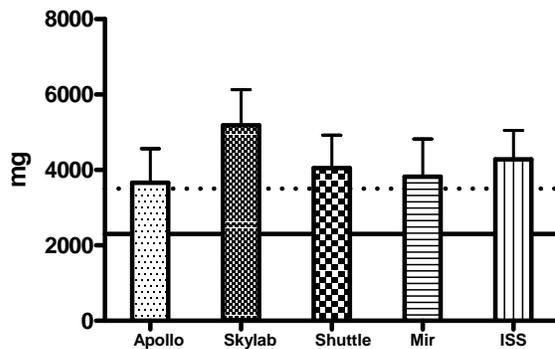
**Figure 24.** Plasma sodium of Skylab crewmembers (N=9) before, during, and after flight (39).

**Figure 25.** Plasma chloride of Skylab crewmembers (N=9) before, during, and after flight (39).



**Figure 26.** Serum sodium (left panel) and chloride (right panel) of Shuttle crewmembers (N=2-6) during and after flight, expressed as a percentage change from preflight values (208).

In-flight sodium intakes (Figure 27) during Skylab and Shuttle missions averaged 4-5 g and were not dissimilar from the astronauts' preflight intakes (7). 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 (1, 2, 6, 31). Intakes as high as 7-10 g of sodium per day have been observed. Sodium homeostasis and blood sodium levels are maintained during real and simulated spaceflight (200), 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.



**Figure 27.** Inflight dietary sodium intake (mg/d) across space programs.

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 (209-212). Dietary sodium is known to affect calcium homeostasis (212-218). 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 (219). This phenomenon is expressed at high levels of dietary sodium. More than 90% of dietary sodium is absorbed, even when intake is high (220). 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) (221). Cyclic AMP also influences reabsorption of sodium (222).

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  $1.8 \pm 0.4\%$  of the total dose, and when smaller amounts of sodium were ingested (50 mmol/d), an average of  $6.0 \pm 1.0\%$  was excreted in the feces.

Salt loading alone increases intestinal calcium absorption. In hypoparathyroid patients, dietary salt increased intestinal calcium absorption in one study by Meyer (223) but not in another study by Breslau (224). 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 (225, 226). Ginty et al (225) 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 (226) 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 (211) reported that postmenopausal women ingesting 300 mmol sodium per day for 7

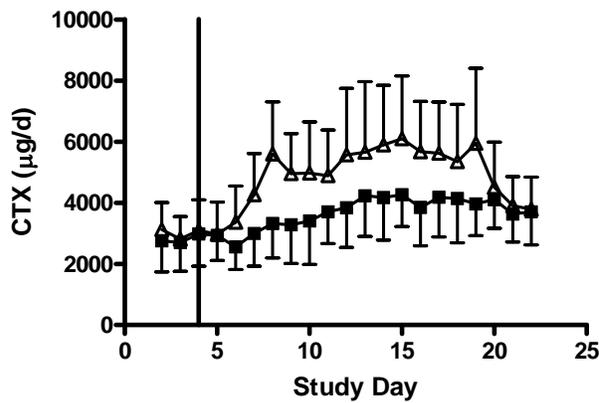
days had greater excretion of bone resorption markers than those ingesting 50 mmol sodium per day. These differences were not observed in a premenopausal group (211). These results suggest that bone resorption is increased in situations where the adaptive responses of bone are limited or altered, as 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 (227-233), and they further suggest that restriction of dietary sodium will reduce bone resorption (234). In a review of the interactions between dietary salt, calcium, and bone, Massey and Whiting (232) 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 (101, 235).

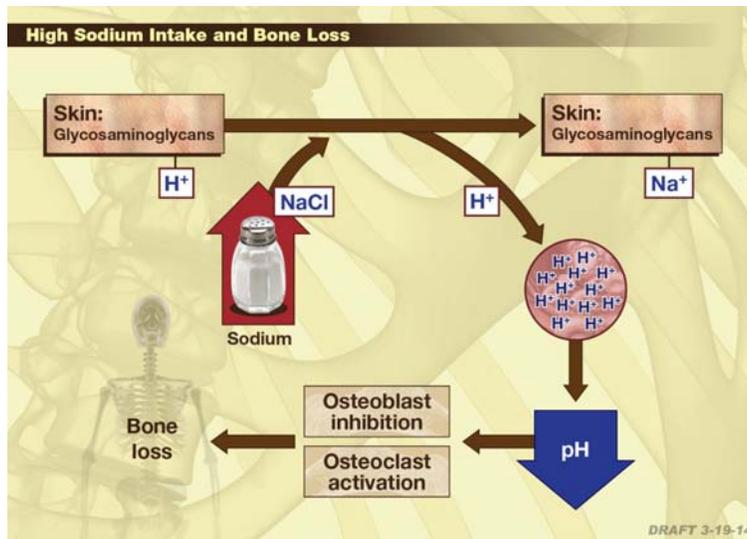
Massey and Whiting (232) found that the effect of excessive salt intake on bone loss is modulated in specific subpopulations. For example, people who tend to form renal calcium stones are more responsive to changes in dietary salt than are non-stone formers. Although sodium intakes of stone formers are typically similar to those of controls (236, 237), the detrimental effects of high sodium intakes on renal stone risk have been well documented (227, 233, 235). 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 (238).

Work by Goulding (209, 210) and Matkovic (239) 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 (240). 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 (241, 242).

Dietary sodium also seems to exacerbate the calciuric responses to musculoskeletal unloading in weightlessness. 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 (243). 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 (Figure 28), through a mechanism mediated by acid-base balance (241, 242). Figure 29 shows a proposed mechanism for the effects of dietary sodium on bone (6). Additionally, the mechanism could be that metabolic acidosis causes an increase in urinary corticosterone (244). Increased sodium intake and consequent low-grade metabolic acidosis caused an increase in bioactive glucocorticoids (203, 245). In turn, the increase in glucocorticoid concentration caused rapid bone loss (246-249). Applying an alkaline salt together with high sodium intake (245) 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 (250), including the role of sodium in bone loss (212, 251, 252).



**Figure 28.** Urinary C-telopeptide (CTX) excretion in bed rest subjects on high-sodium (open triangles) or low-sodium (filled squares) diets. Bed rest began on day 4, indicated by the vertical line. Adapted from (241, 242).



**Figure 29.** Proposed mechanism of the effects of high dietary sodium on bone loss (6).

In the U.S., the recommendation for adequate intake of sodium is 1.5 g/d for men and women ages 19-50 years and 1.3 g/d for men and women ages 51-70 years (190). The current documented spaceflight requirement for dietary sodium is 1500-2300 mg/d for both women and men.

### **3. Risk**

High sodium intakes during spaceflight 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 sodium or a deficiency of this electrolyte during flight could lead to hyponatremia, hypotension, and even death.

### **4. Gaps**

Further research is required to investigate potential effects of high sodium intake during spaceflight, 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, but 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 spaceflight interact, and study of the interaction may produce a dietary countermeasure.

## **H. Potassium**

### **1. Background**

As the most plentiful intracellular cation, potassium has a significant role in several physiological processes (193). 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 (253). Potassium metabolism that is disordered because of excessive or deficient circulating levels has negative consequences for cardiac, muscle, and neurological function.

Total body potassium averages 45 mmol/kg body weight, totaling about 3150 mmol (1230 g) of potassium in a reference 70-kg person. Two percent of body potassium (~60 mmol) is distributed in the extracellular fluid, and intracellular fluid levels are typically maintained at 140-150 mmol/L).

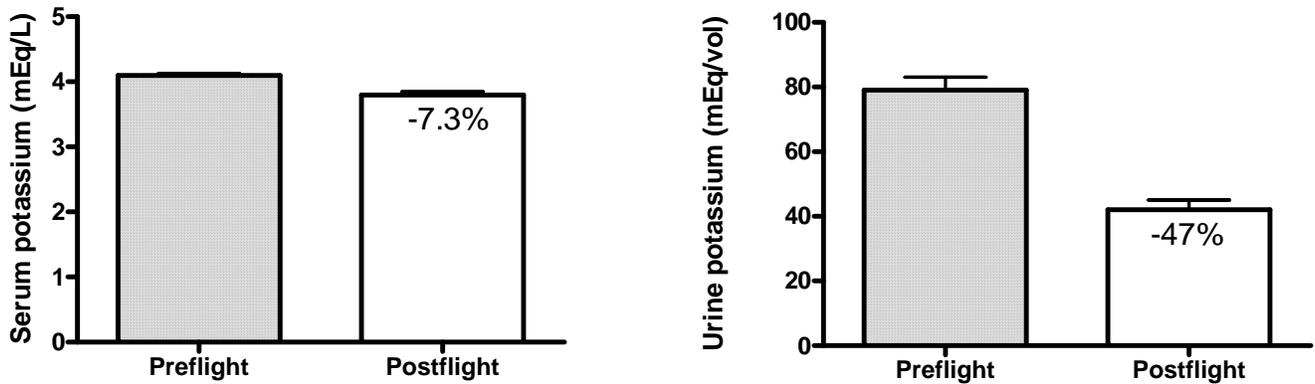
Potassium levels cannot be maintained at intakes under 10-20 mmol/d (254). Moderate depletion of potassium in humans is associated with clinically significant impaired active relaxation of the left ventricle (255). In the referenced study, healthy adults were placed on a potassium-depletion diet for 7 days. At the end of 7 days, isovolumic relaxation time and deceleration time of flow through the mitral valve were significantly increased.

Increased levels of urinary potassium may be related to muscle disuse atrophy and inadequate intake during spaceflight (200).

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

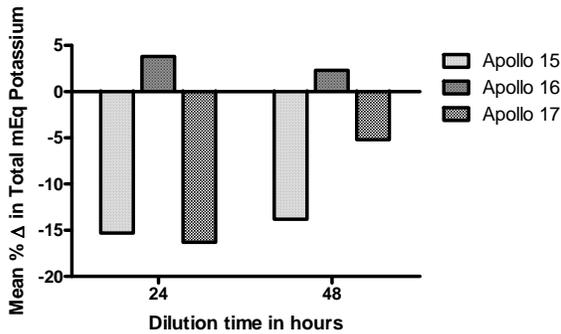
## 2. Evidence

Both serum and urinary levels of potassium decreased after spaceflight in the Apollo program (Figure 30) (104), and there is evidence of a similar decrease on Skylab (Figure 31) and Shuttle (Figure 32) missions (39, 208).

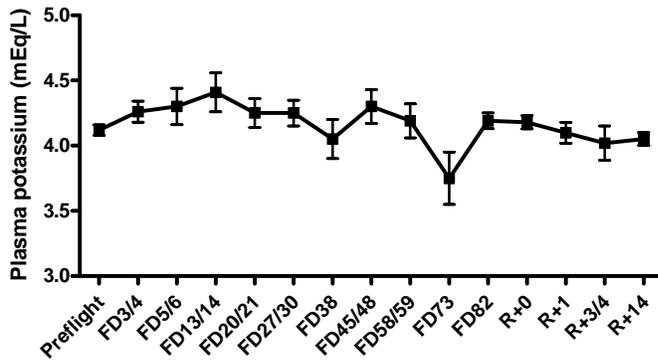


**Figure 30.** Serum (N=33) and urinary (N=30) potassium of Apollo crewmembers. Numbers in bars represent the percentage change from preflight values. Adapted from (104).

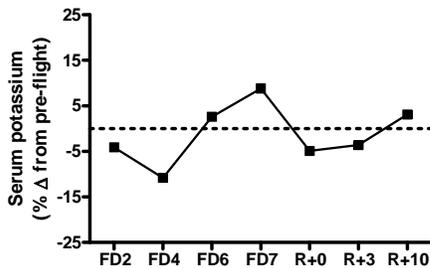
Potassium loss (both total body potassium and exchangeable potassium) was observed in Apollo crewmembers (Figure 31) (104).



**Figure 31.** Exchangeable potassium of Apollo 15, 16, and 17 crewmembers after flight, as the percentage change from preflight values (104).



**Figure 32.** Plasma potassium of Skylab crewmembers (N=9) before, during, and after flight (39).



**Figure 33.** Serum potassium of Shuttle crewmembers (N=2-6) during and after flight, expressed as the percentage change from preflight values (208).

Excess dietary sodium has been shown to be potassium-depleting during bed rest (Heer et al, unpublished observations).

The current documented spaceflight requirement for potassium is 4.7 g/d, the same as the U.S. recommendation for adequate intake of potassium for men and women aged 19-70 years (190).

### 3. Risk

The loss of lean body mass, along with high sodium intake, may result in potassium depletion. The implications of potassium depletion for cardiac, musculoskeletal, and other systems are profound.

### 4. Gaps

The relationship between bone health and the protein:potassium ratio is currently being investigated on ISS. The role of potassium in cardiovascular health during flight requires additional study.

## **VI. Gap N3.2: Determine the micronutrient requirements for spaceflight.**

### **A. Vitamin A**

#### **1. Background**

One of the vitamins important to vision health is vitamin A. Vitamin A and the structurally related 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 (256).

Beyond its essential role in the visual process, vitamin A is directly involved in gene expression, reproduction, embryonic development, and immunity. Vitamin A and  $\beta$ -carotene serve as biological antioxidants and have been shown in multiple studies to reduce the risk of cancer and coronary heart disease (257, 258). Vitamin A also plays a role, albeit sometimes indirectly, in the function of almost all of the body's organs (259).

Vitamin A is one of the fat-soluble vitamins. Less concern is expressed about the adequacy of fat-soluble vitamin intake than about the intake of water-soluble vitamins because the body can store larger quantities of fat-soluble vitamins. However, recent findings about previously unknown functions of some of these vitamins, as well as unique aspects of spaceflight, provide specific challenges for maintaining optimum status of these nutrients.

Vitamin A is a general term for a family of fat-soluble compounds that are structurally similar to retinol and share its biological activity. Among these are retinol,  $\alpha$ -carotene,  $\beta$ -carotene, and retinyl palmitate. Trans-retinol is the primary biologically active form of vitamin A. Many carotenoids, including  $\beta$ -carotene, can be converted to trans-retinol and thus contribute to vitamin A activity. Collectively, these carotenoids are termed provitamin A carotenoids and are measured in retinol equivalents (REs).

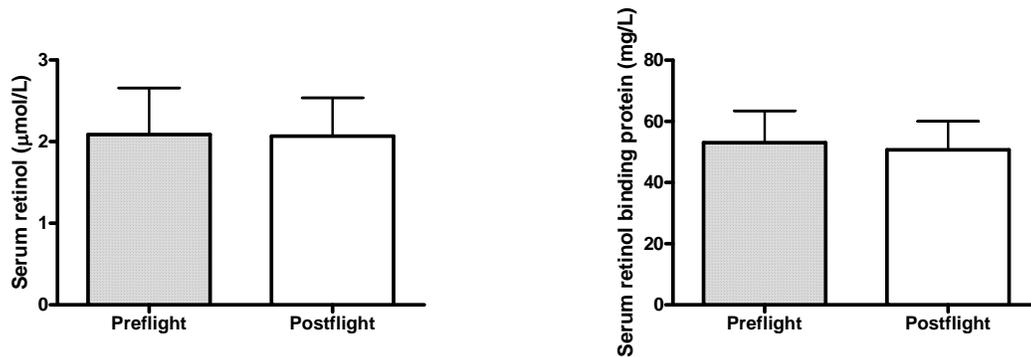
Vitamin A is stored mainly (80%) in the liver, with the remainder stored in peripheral organs and tissues. Total body stores range from 1.05 to 3.14 nmol (300-900 mg) in normal adults (260). Liver stores of vitamin A are severely depleted when levels are less than 20  $\mu$ g (261). A study of vitamin A depletion in baboons showed a 59% decrease in hepatic vitamin A after 4 months of a chronic ethanol diet (262). After 24-48 months, the researchers found a 95% decrease in hepatic vitamin A stores, which was accompanied by fibrosis and cirrhosis of the liver. Alcoholism is often associated with vitamin A deficiency because retinol and ethanol are competing substrates for the same enzymes (263).

Deficiency of vitamin A leads to xerophthalmia, loss of appetite, drying and keratinization of membranes, infection, or even death. Acute toxicity of vitamin A leads to nausea, vomiting, headache, blurred vision, and muscular incoordination. Chronic toxicity of vitamin A leads to rapid reduction in bone mineral density, liver abnormalities, or even death.

## 2. Evidence

Oxidative stress is increased during spaceflight, 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 spaceflight; 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 amounts on the order of twice the recommended daily intake, has been shown to increase bone resorption and fracture risk (264-267). Furthermore, supplementation with  $\beta$ -carotene should be done with caution (either alone or with vitamin A, or in combination with vitamin E), because of the unanticipated outcome of an increased risk of lung cancer in smokers (268, 269). This increased risk among smokers might be related to pro-oxidant actions of  $\beta$ -carotene in the lung.

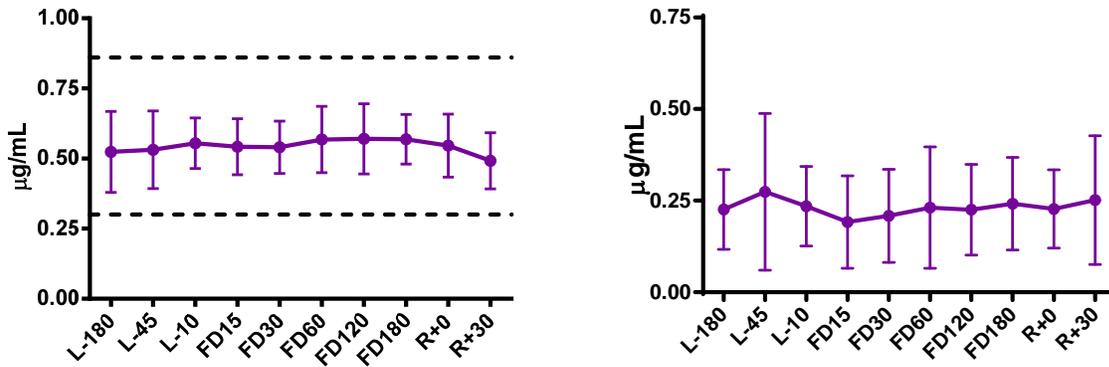
When considering pre- and postflight spaceflight data, a significant interaction occurs between the effects of landing site and spaceflight on serum levels of both retinol and retinol-binding protein (Figure 34) (31). Russian landings are different from U.S. landings in that blood samples are usually collected several hours later because of the logistics of the landing site.



**Figure 34.** Serum retinol and retinol-binding protein in ISS crewmembers before and after long-duration spaceflight. Adapted from (31).

Serum retinol decreased from  $0.73 \pm 0.17$   $\mu\text{g}/\text{mL}$  before flight to  $0.59 \pm 0.13$   $\mu\text{g}/\text{mL}$  after landing when landings were in Russia, and increased from  $0.52 \pm 0.09$   $\mu\text{g}/\text{mL}$  to  $0.63 \pm 0.12$   $\mu\text{g}/\text{mL}$  when landings were in the U.S. Similarly, retinol-binding protein decreased from  $61.4 \pm 5.6$  to  $50.92 \pm 8.41$   $\text{mg}/\text{L}$  when landings were in Russia, and increased from  $49.2 \pm 9.2$  to  $53.0 \pm 8.7$   $\text{mg}/\text{L}$  when landings were in the U.S. These differences associated with 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 35 shows that no significant changes in retinol or  $\beta$ -carotene occur during spaceflight.



**Figure 35.** Serum retinol (left) and  $\beta$ -carotene (right) in ISS crewmembers before, during, and after long-duration spaceflight. Dashed lines represent the normal range for retinol. Figure from (6).

In addition to the vision-related changes observed in some astronauts after long-duration spaceflight, several studies have confirmed that astronauts and cosmonauts have an increased risk of cataract formation after spaceflight (270-273). Cucinotta et al (271) 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 (272, 274). 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 (272). 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  $\beta$ -carotene and lycopene intake had a protective effect for some types of cataracts in astronauts (272). 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 (275). A recent meta-analysis provided similar results supporting an inverse association with  $\alpha$ -carotene (and vitamin E, lutein, and zeaxanthin) and age-related cataract (276).

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 spaceflight 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.

### **3. Risk**

Oxidative stress is increased during spaceflight, which could affect cardiovascular health and cancer risk. Vitamin A status may play a critical role in maintaining antioxidant health during spaceflight.

As with many antioxidants, the desire to supplement with high doses in the hope of staving off one disease is high but can be unwarranted and potentially counterproductive; for example, excess vitamin A, in amounts on the order of twice the recommended daily intake, has been shown to increase bone resorption and fracture risk (264-267).

In the U.S., the recommended dietary allowance for vitamin A is 900  $\mu\text{g RE/d}$  for men aged 19 and older and 700  $\mu\text{g RE/d}$  for women aged 19 and older (277). Upper limits also exist for vitamin A (3000  $\mu\text{g RE/d}$ ), and  $\beta$ -carotene supplementation is advised only in situations where there is a risk of vitamin A deficiency. The current documented spaceflight requirement for vitamin A intake is 700-900  $\mu\text{g/d}$ .

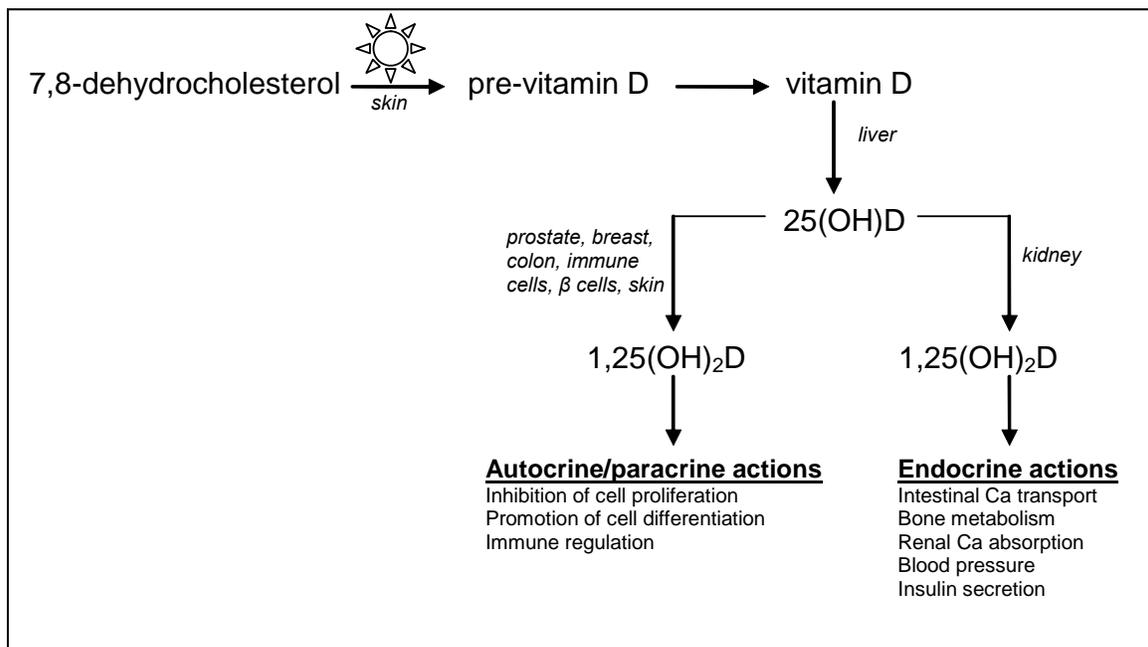
### **4. Gaps**

Vitamin A content and stability in the space food supply should be determined. The role of vitamin A as an antioxidant has not been investigated.

## B. Vitamin D

### 1. Background

The best-understood role of vitamin D is its involvement in calcium metabolism. One of the major functions of this vitamin—its calcitropic function—is to maintain normal blood levels of calcium and phosphorus. A component of sunlight acts on 7,8-dehydrocholesterol in the skin to form pre-vitamin D, which is converted to vitamin D. The liver converts vitamin D to 25-hydroxyvitamin D, which is typically the gold standard measurement for determining vitamin D status (Figure 36). In the kidney, 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitamin D, which is transported from the kidney systemically to target organs. Classic target organs include bone, intestine, and kidney.



**Figure 36.** Vitamin D synthesis, activation, and catabolism. Adapted from (278).

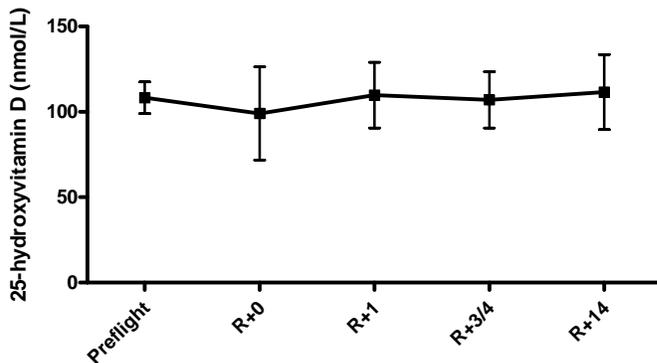
The Institute of Medicine (IOM) conducted an extensive review of the literature in 2010 and increased the recommended dietary allowance (RDA) for vitamin D to 600 IU/d for healthy males and females 9-70 years old, and 800 IU/d for those older than 70 years (279, 280). The main factors that were taken into account were changes in bone density and fracture risk. The IOM review 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 low 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 (281-286). The non-calcitropic 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 (287-289). 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 (290). Numerous tissues are affected by vitamin D status because their cell nuclei contain receptors for 1,25-dihydroxyvitamin D (284). Some of these tissues are adipose tissue, bone marrow, brain, breast, cancer cells, cartilage, lung, muscle, ovary, placenta, prostate, stomach, testis, thymus, and uterus (291).

People who are normally exposed to sunlight make vitamin D in their skin (Figure 36). Ultraviolet B (UV-B) light, a component of sunlight, converts 7-dehydrocholesterol to 25-hydroxyvitamin D<sub>3</sub> in the skin (292). 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 (293-295).

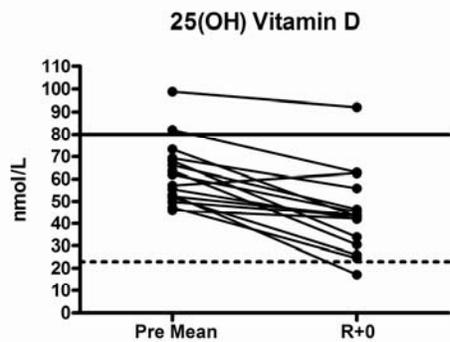
### **Evidence**

Spacecraft typically shield crewmembers from the UV-B radiation that forms 25-hydroxyvitamin D<sub>3</sub> in the skin (the only exception to shielding being the rare quartz windows). Crewmembers on the longest Skylab mission (Skylab 4, 84 days), but not the shorter missions (28 and 59 days), had decreased serum 25-hydroxyvitamin D at landing (39) despite taking daily vitamin D supplements (Figure 37). Provision of 400 IU/d vitamin D supplements did not prevent a decrease in vitamin D status during flight in ISS crewmembers (31).



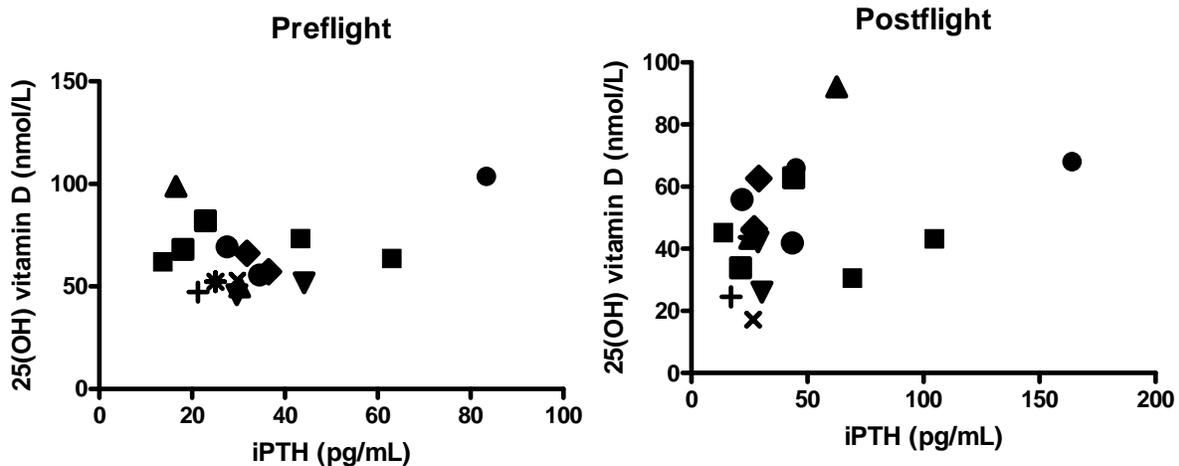
**Figure 37.** Plasma 25-hydroxyvitamin D of Skylab crewmembers (N=9) before and after flight (39).

Decreased vitamin D status is one of the most striking nutritional changes that has occurred during spaceflight (30, 31). The mean preflight serum 25-hydroxyvitamin D for early U.S. ISS crewmembers is  $62 \pm 14$  nmol/L (Figure 38). In several studies, crewmembers on the Russian space station Mir had serum 25-hydroxyvitamin D<sub>3</sub> concentrations that were 32% to 36% less during and after long-duration (3- to 4-month) missions than before the missions (30, 63). ISS astronauts had serum 25-hydroxyvitamin D concentrations that were typically 25% to 30% lower after 4- to 6-month spaceflights (31), and in several ISS crewmembers, serum 25-hydroxyvitamin D decreased to levels considered clinically significant (Figure 38) (31).



**Figure 38.** Serum 25-hydroxyvitamin D concentrations before and after 4- to 6-month spaceflights on the International Space Station. Each line represents one crewmember. The “Pre Mean” point on each line is the average of data collected about 6 months and 6 weeks before launch. R+0 = Recovery plus zero days, that is, landing day. These samples are typically collected 2-8 hours after landing. The dashed line shows the (outdated) lower limit of normal range, and the solid horizontal line shows what many considered to be the “optimal” concentration of vitamin D in the circulation. From (1, 31).

Another important observation from ISS nutritional status assessment was related to the relationship between parathyroid hormone (PTH) and 25-hydroxyvitamin D before and after ISS missions. Before launch, 25-hydroxyvitamin D was inversely correlated with PTH ( $r = -0.72$ ,  $P < 0.05$ ) (Figure 39), but this relationship was not evident after landing, suggesting that the body’s normal response to changes in vitamin D was altered (31).



**Figure 39.** Serum 25-hydroxyvitamin D and parathyroid hormone (PTH) concentrations before (average of data from samples collected about 6 months and 6 weeks before launch) and after (landing day, typically collected 2-8 hours after landing) 4 to 6-month spaceflights on the International Space Station. Each symbol represents one crewmember. From (1, 31).

Results from ground-based studies of bed rest subjects (296) and subjects living in closed-chamber facilities for extended periods suggest that these subjects are also at high risk of having vitamin D deficiency (29). Ground-based models with limited sunlight exposure are valuable for performing vitamin D supplementation trials.

Perhaps an ideal ground-based model for individuals lacking UV-B light exposure is the Antarctic, where winter levels of UV-B radiation are essentially zero. We recently completed a

study at McMurdo Station, Antarctica, to determine the daily 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 UV-B exposure.

The environment in the Antarctic is quite unique. Seasonal changes in UV-B light exposure are more extreme than in any other part of the world. The sun does not rise for 42 days during winter (June 1 – July 12), and the sun does not set for 60 days during the summer months (Nov 22 – Jan 20). During the Antarctic winter, scientists and visitors are typically isolated, and no fresh fruits or vegetables are available. As a result of close quarters and limited food choices throughout the year, most scientists at a particular research station have homogeneous food intakes and physical activities. Not only is Antarctica a good model for studying vitamin D metabolism because of the limited sunlight exposure, but also the Antarctic science station model has been used successfully as a ground-based analog for spaceflight in studies of behavior, immune response, and latent virus reactivation (297-299).

It is well documented that vitamin D status is decreased among subjects who live in Antarctica for an extended period (300-304). One research group examined 25-hydroxyvitamin D status of 31 members of an Antarctic wintering team who stayed at the Japanese Antarctic station, Syowa, for 14 months. For one week in May and one week in July, food items were weighed before they were cooked and vitamin D intake was estimated to be 488 IU/d. Serum 25-hydroxyvitamin D was lowest during April – October ( $\sim 19.0 \pm 4.4$  ng/mL, or  $\sim 47.5$  nmol/L) (301). Because there was no source of UV-B during the winter months, it can be concluded from this study that 488 IU/d is not enough vitamin D to prevent a decrease in serum 25-hydroxyvitamin D levels. Olivieri and colleagues studied bone metabolism in wintering individuals for 1 year and found that the mean 25-hydroxyvitamin D level in Argentine Antarctic wintering researchers was 10 ng/mL (25 nmol/L) during the winter months (300). On a different 1-year expedition to Antarctica, both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were significantly decreased by about 25% during the winter months (303).

To our knowledge, only one supplementation trial has successfully maintained serum 25-hydroxyvitamin D in subjects living in Antarctica during the summer (302). Despite the sunlight in the summer, temperatures still keep skin exposure to the sun at a minimum. Twenty-two healthy males were randomly divided into two groups: individuals supplemented with 1000 IU vitamin D<sub>3</sub> per day and non-supplemented controls. Both groups ingested less than 500 mg/d calcium. Blood was sampled at intervals of 22 days during the Antarctic summer months. Serum 25-hydroxyvitamin D levels were significantly decreased in the control group but did not change in the supplemented group. In the latter group, however, a significant decrease in PTH occurred. This study showed that 1000 IU/d vitamin D was enough to maintain vitamin D status during the summer months in Antarctica.

Although these data suggest that 1000 IU/d vitamin D may be enough to offset changes in vitamin D status in subjects with limited sun exposure such as astronauts, they shed no light on the efficacy of 1000 IU/d vitamin D during the winter months in Antarctica, the time when Antarctica most closely models spaceflight. In the winter months, there is little to no UV-B exposure. The mean monthly amount of total UV-B radiation at the Syowa station in Antarctica is greatest in December (49,540 J/m<sup>2</sup>) and smallest in June and July (0 J/m<sup>2</sup>) (301). In Tsukuba, Japan, which is similar in latitude to the Midwest in the United States, the maximum total daily UV-B is about 20,550 J/m<sup>2</sup> and the lowest is about 5,010 J/m<sup>2</sup> (301). Astronauts aboard the International Space Station and those who will travel to Mars will not be exposed to any UV-B radiation because spacecraft successfully block UV-B radiation (305). The zero UV-B radiation

exposure and zero availability of fresh fruits and vegetables due to forced isolation make the winter months in Antarctica a better model for spaceflight than the summer months, when many researchers work outdoors (temperatures range from -5 to +6 °C).

In addition to a decrease in vitamin D status, wintering residents in Antarctica show signs of impaired cognitive function and changes in psychosocial behavior (306, 307). Although there is some evidence that a vitamin D deficiency may be related to changes in cognitive function (308), further research is needed to determine whether these changes are related to nutritional issues or other reasons.

Glerup and colleagues compared vitamin D status in sunlight-deprived individuals (veiled Arab women living in Denmark, veiled Danish Muslim women, and non-veiled Danish women) and found a severe vitamin D deficiency among veiled Arab women (serum 25-hydroxyvitamin D was  $7.1 \pm 1.1$  nmol/L) (309). Twenty-six percent of the veiled Arab women reported a change in gait compared with 9% of non-veiled Danish controls, and muscle pain was felt by 88% of Arab women. These women had a very low vitamin D intake (1.04 µg/d) and limited sun exposure. Veiled Danish Muslims had a high vitamin D intake [13.53 µg/d (~600 IU/d)] but limited sun exposure, and they were still vitamin D deficient (serum 25-hydroxyvitamin D was  $17.5 \pm 2.3$  nmol/L). These data suggest that 600 IU/d vitamin D is not enough to maintain 25-hydroxyvitamin D levels in individuals with little or no sun exposure. In another study with 51 submariners on deployment, 400 IU vitamin D was not enough to prevent a decrease in vitamin D status after 76 days (310).

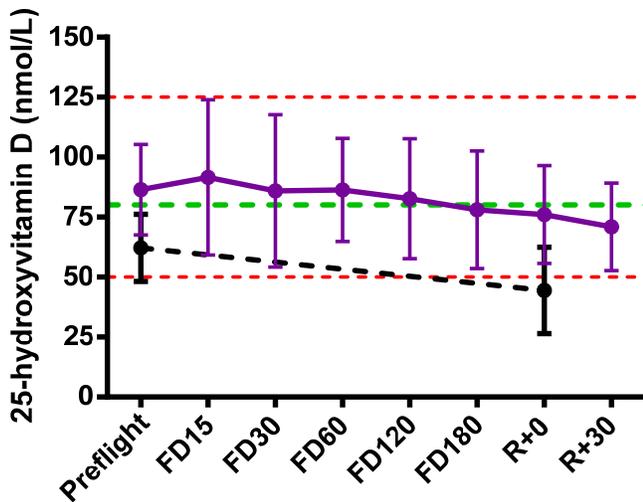
Before 2006, when ISS crews were first provided 400 IU vitamin D/d, it was well documented that vitamin D status (serum 25-hydroxyvitamin D) decreased after long-duration spaceflight (30, 31, 39, 63). The absence of UV-B light during spaceflight diminishes vitamin D stores in the body, as observed during the 84-day Skylab mission (39), space station Mir missions (30, 63), and early ISS expeditions (31). Despite the reported use of vitamin D supplements by some of the astronauts on early ISS expeditions (average supplement use was  $3.0 \pm 2.8$  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 supplement 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 spaceflight (Figure 40) (41). Furthermore, recent data show that crewmembers who exercised with the Advanced Resistance Exercise Device, maintained energy intake at recommended levels, and took 800 IU/d vitamin D during flight maintained bone mineral density during 4- to 6-month spaceflights (41).

An ideal ground-based model for individuals lacking UV-B light exposure is the Antarctic, where winter levels of UV-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 UV-B exposure, without increasing risks of hypercalcemia (311, 312). 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, safe, and can maintain vitamin D status for 3 to 6 months even in environments with no UV light exposure (311-313). This recommendation is in line with the recent Institute of Medicine recommendations for vitamin D intake for North Americans

(279).

One of the vitamin D studies conducted at McMurdo Station showed an interactive effect of serum cortisol and vitamin D status on immune function (312). In that study, subjects with higher serum cortisol and lower vitamin D status had greater 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 40.** Pre- and postflight data from testing required for medical operations show that vitamin D status decreased after long-duration spaceflight, 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<sub>3</sub> to maintain status during long-duration spaceflight (N=26). Red lines depict Institute of Medicine-defined lower acceptable limits (with respect to bone health), and upper advised limit (279). 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 (314).

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 (30, 63). This is likely the result of the increased release of calcium from resorbed bone (see subsection N, “Calcium,” in this section), 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, or 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 PTH, and no effect on bone markers (315). In a similar 40-week trial of 1000 IU of vitamin D<sub>2</sub> or D<sub>3</sub> (2 groups), neither had an effect on bone markers (316). 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.

### **3. Risk**

The current documented spaceflight requirement for dietary intake of vitamin D is 25 µg per day. Because astronauts in space are shielded from sunlight, considering them to be in a high-risk group seems appropriate. It is currently recommended that ISS crewmembers consume 800 IU of vitamin D per day during long-duration spaceflight. 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 (317-320), 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 (313). In that study, a daily dose of 2000 IU or a single weekly dose of 10000 IU did not increase the incidence of hypercalciuria.

### **4. Gaps**

Vitamin D levels in the food system need to be determined, and the stability of vitamin D in the food system needs to be investigated. The interaction of vitamin D, stress, and immune system function during spaceflight requires further study. Monitoring of vitamin D status should continue, along with efforts to inform stakeholders about the importance of adequate vitamin D status and risks of vitamin D toxicity.

## **C. Vitamin E**

### **1. Background**

Vitamin E is a lipid-soluble, chain-breaking antioxidant found in body tissues, and it is also the first line of defense against lipid peroxidation reactions. Eight naturally occurring compounds have vitamin E activity: four tocopherol derivatives ( $\alpha$ -,  $\gamma$ -,  $\delta$ -, and  $\beta$ -tocopherol) and four tocotrienol derivatives ( $\alpha$ -,  $\gamma$ -,  $\delta$ -, and  $\beta$ -tocotrienol) (321). The tocopherols that are most abundant in biological systems are  $\alpha$ - and  $\gamma$ -tocopherol, but small amounts of  $\delta$ -tocopherol and  $\alpha$ -tocopheryl quinone are also present. About 90% of the tocopherol found in human plasma is in the form of  $\alpha$ -tocopherol (322).

Vitamin E helps protect cell membranes in the early stages of free-radical attack because of its free-radical-quenching activity. Free radicals attack polyunsaturated fatty acids found in membrane phospholipids, causing damage to cellular membranes and possibly cell death. The interception of a free radical by vitamin E produces a tocopheroxyl radical that can be reduced by vitamin C or another reducing agent to return vitamin E to its reduced state. The extent of regeneration and recycling of vitamin E in human tissue has not been well established (321). Vitamin E has important roles related to immune system function (see Gap N7.5 below).

Vitamin E is stored mainly in adipose tissue and is also found in phospholipid membranes. Results of studies conducted to determine vitamin E tissue levels have shown that tissue  $\alpha$ -tocopherol concentrations are largely reflected by changes in plasma  $\alpha$ -tocopherol concentrations (323).

Vitamin E deficiencies in humans are rare; however, fat malabsorption syndromes, genetic abnormalities, and protein-energy malnutrition are specific cases when a vitamin E deficiency is likely to occur (324). Symptoms include neurological problems associated with nerve degeneration in the extremities (322). Vitamin E depletion has been detected when markers of lipid peroxidation were elevated. However, the lowering of levels of these lipid peroxidation markers has not been shown to have any health benefits, and, therefore, they have not been used to establish  $\alpha$ -tocopherol requirements.

Deficiency of vitamin E leads to neurological disorders, hemolytic anemia, retinopathy, abnormal platelets and lymphocytes, or even death. Toxicity of vitamin E from naturally occurring sources has not been shown to occur.

### **2. Evidence**

After ISS crewmembers had spent 4 to 6 months in space, their plasma  $\gamma$ -tocopherol was 50% less than preflight levels (31). No change in  $\alpha$ -tocopherol occurred in these subjects. Preliminary data collected during spaceflight show similar changes, that is, a decrease in circulating  $\gamma$ -tocopherol and no change in circulating  $\alpha$ -tocopherol (6). This is true for circulating tocopherols, whether they are normalized to lipid concentrations or not. The change in  $\gamma$ -tocopherol likely reflects differences in sources of vitamin E from the space food system. Animal hindlimb unloading studies have shown that supplemental vitamin E can have a positive influence on bone, potentially through antioxidant properties (325).

The current documented spaceflight requirement for dietary intake of vitamin E is 15 mg/d for men and women, the same as the U.S. recommendation for adequate intake of vitamin E. No

upper limit has been established because the highest level of daily intake is not likely to pose serious health risks to the majority of individuals (322). Although no striking changes occurred in plasma vitamin E concentrations, the spaceflight menus provide only about 60% of the documented requirement for vitamin E.

### **3. Risk**

Oxidative stress can increase in microgravity and high-radiation environments (326-328), 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 (329), and it may be necessary to provide enough vitamin E for astronauts' blood levels of the vitamin to be higher during spaceflight than on Earth. However, knowledge gaps weaken the evidence for use of vitamin E as a countermeasure (see below).

### **4. Gaps**

Vitamin E content of space foods, along with the stability of vitamin E in these foods, needs to be determined. After learning about the promising antioxidant effects of supplemental vitamin E, many people on Earth did not hesitate to take vitamin E supplements to prevent cancer. The protective effects were not borne out in controlled studies, highlighting the difficulties of defining a specific antioxidant countermeasure for space travelers without the luxury of having data from epidemiological studies to provide an evidence base for spaceflight.

## **D. Vitamin K**

### **1. Background**

Vitamin K occurs naturally in two forms: phylloquinone (vitamin K<sub>1</sub>) and menaquinone (vitamin K<sub>2</sub>). Menaquinones are produced by bacteria, while phylloquinone is synthesized in plants. Phylloquinone represents the main source of dietary vitamin K in Western countries (330, 331).

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 post-translational synthesis of  $\gamma$ -carboxyglutamic acid (GLA) (332). Gamma-carboxyglutamic acid is common to all vitamin K-dependent proteins, and its role is related to increasing the affinity of the proteins for calcium (333). Vitamin K-dependent proteins include blood coagulation proteins (prothrombin; factors VII, IX, and X; and proteins C and S) and bone proteins (osteocalcin, matrix GLA protein, and protein S).

The main storage depot for vitamin K in the body is the liver. Large amounts of vitamin K are also present in cortical and trabecular bone (334). However, vitamin K stores are very small compared to those of other fat-soluble vitamins, and hepatic vitamin K is rapidly depleted when dietary vitamin K is restricted (335).

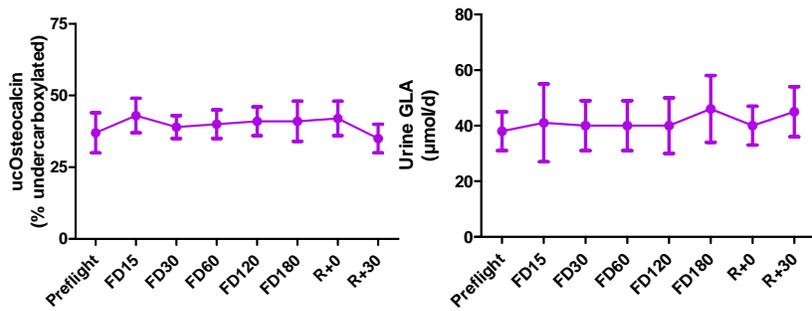
Anticoagulants such as warfarin, a coumarin-based anticoagulant, are administered to create a partial vitamin K deficiency to reduce risks of abnormal blood clotting (336). Dosing with warfarin must be closely monitored for optimal efficacy and safety. Generous or poor intake of vitamin K can interact with the actions of warfarin to yield non-therapeutic anticoagulation or life-threatening hemorrhagic complications (337, 338).

### **2. Evidence**

Data from 11 U.S. astronauts on ISS Expeditions 1-8 (mission durations of 128-195 days during 2000-2004) revealed that on landing day, their serum phylloquinone (vitamin K<sub>1</sub>) was 42% lower than it was before flight (Figure 41), whereas urinary  $\gamma$ -carboxyglutamic acid did not change (31). In one study, undercarboxylated osteocalcin was elevated (a sign of vitamin K insufficiency) as early as the 8<sup>th</sup> day of spaceflight and remained high during 21- and 180-day missions (339). Studies on the EuroMir 95 mission showed that markers of vitamin K status were decreased after 12.5 weeks of spaceflight, and vitamin K supplementation (10 mg/d for 6 weeks) reversed these effects (340). Vitamin K supplementation elevated urinary  $\gamma$ -carboxyglutamic acid and decreased urinary undercarboxylated osteocalcin (a bone protein), suggesting that vitamin K status was lower during spaceflight and was improved by supplementation (339, 340).

Monitoring vitamin K status during flight for the Nutrition Status Assessment Supplemental Medical Objective project (aka, Nutrition SMO) has documented a lack of evidence that vitamin K status is decreased during spaceflight, a finding earlier reported from a single case study on Mir (339, 340). Nutrition SMO data from the first 15 participating crewmembers showed no major changes in phylloquinone, urinary GLA, or undercarboxylated osteocalcin (Figure 41) (341). This was an important finding, to document that a vitamin K countermeasure to mitigate a vitamin K deficiency during spaceflight is not needed and therefore likely would not have an

effect on bone.



**Figure 41.** Effect of long-duration spaceflight on vitamin K status of 15 ISS crewmembers. Undercarboxylated osteocalcin (left) and urinary  $\gamma$ -carboxyglutamic acid (GLA, right) are shown here. There were no significant changes as a result of spaceflight (341).

The current documented spaceflight requirements for dietary intake of vitamin K are 90 and 120  $\mu\text{g}/\text{d}$  for women and men, respectively. These are the same as the U.S. adequate intake recommendations for vitamin K (277). No upper limit has been established.

### 3. Risk

Decreased vitamin K status has serious implications for spaceflight because it is related to bone health. Although spaceflight data, including data from Mir (339) and ISS crewmembers (31), suggest that vitamin K status during long-duration spaceflight was suboptimal, more recent data do not support this contention (6, 341). Elevated undercarboxylated osteocalcin has been associated with increased fracture risk in certain populations, and evidence exists that vitamin K antagonists increase the risk of fracturing vertebrae and ribs in a time-dependent manner (342, 343).

### 4. Gaps

Deficiency of vitamin K is not common in adults, as the intestinal microflora synthesize vitamin K. The reliability of this source of vitamin K during flight is unknown, and expert panels have recommended having higher intake requirements because of this uncertainty (97). It has been hypothesized that microflora production of vitamin K may be altered in space, but few or no data are available to support this. The vitamin K content in the space food system and its stability should be determined.

Water-soluble vitamins are a key concern for space travelers, given the limited endogenous storage of many of these nutrients. They must be replenished from food that may have been stored for a long time (9-18 months) under suboptimal conditions, including the space radiation environment.

## **E. Folate**

### **1. Background**

Folate is the general term used to describe folic acid and compounds that have activity similar to that of folic acid. 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 (344). 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 (344).

Deficiency of folate leads to megaloblastic anemia. Low folate intake will cause red blood cell (RBC) 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 (345). Folate deficiency in humans has been described as a 4-stage process (346, 347), including changes in serum folate (Stage 1), changes in RBC 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. Radiation exposure and inadequate dietary consumption can lead to inadequate intake of folate (348).

### **2. Evidence**

Early ISS flight data showed a reduction in RBC folate after long-duration missions (29, 31). Serum folate is variable among crewmembers, but generally does not change during flight (Figure 42). Interestingly, serum folate was lower during spaceflight in crewmembers with vision-related issues (349).

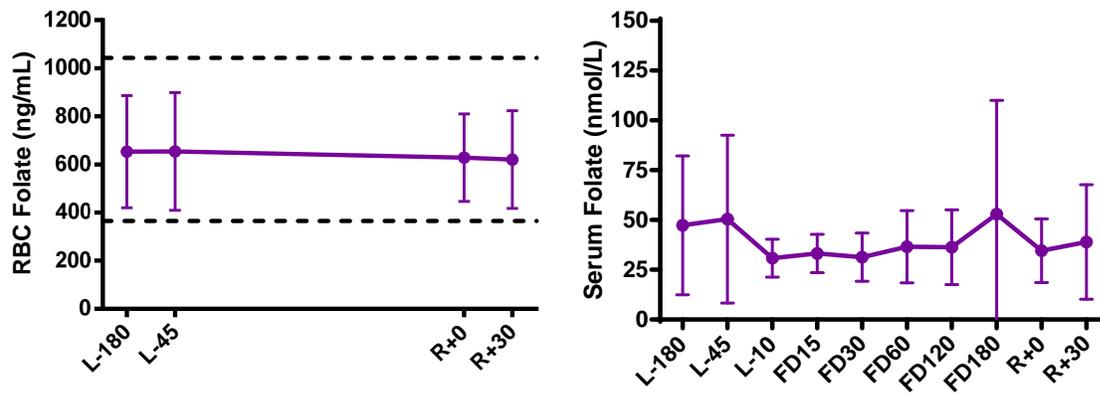


Figure 42. Red blood cell (RBC) (left) and serum folate (right) before, during, and after long-duration spaceflight (data are mean  $\pm$  SD). Note: RBC folate data are not available during flight because of sample processing requirements.

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 (350-353); however, it should be noted that excessive folate supplementation provided no additional benefit (350). Similarly, cell models have shown that folic acid deficiency increases sensitivity to chromosome breakage from ionizing radiation (352). Antioxidant properties of folate have been studied, and folate was found to scavenge a diverse array of reactive oxygen species efficiently (354). Cell models also show the ability of folate to reduce iron toxicity in cases of iron overload, by oxidizing free or chelated iron (354). 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 (355). Folate status may be even more important during exploration missions than on ISS because of known increases in iron storage during long-duration spaceflight (31) and exposure to ionizing radiation. Recent bed rest studies did not document any change in RBC folate status during or after short (3-week (356)) or long (60-90-day (357)) bed rest. A 14-day exposure to increased atmospheric pressure (i.e., saturation diving) did not significantly affect RBC folate (358).

The current documented spaceflight requirement for folate intake is 400  $\mu\text{g}/\text{d}$  (97). In the U.S., the recommended dietary allowance (RDA) for all individuals aged 14 and older is 400  $\mu\text{g}/\text{d}$  of dietary folate equivalents (DFEs). Using DFEs adjusts for the 50% reduction in food folate bioavailability compared with that of folic acid: 1  $\mu\text{g}/\text{d}$  DFE = 0.6  $\mu\text{g}$  of folic acid from fortified food, or as a supplement taken with meals; 1  $\mu\text{g}$  DFE = 1  $\mu\text{g}$  of food folate = 0.5  $\mu\text{g}$  of a supplement taken on an empty stomach. An upper limit of folate intake during spaceflight is set at 1000  $\mu\text{g}/\text{d}$ .

### **3. Risk**

As with many nutrients, folate deficiency on an exploration mission would be catastrophic. Animal studies have shown that low folate status increases chromosomal damage resulting from radiation exposure (350-353); however, it should be noted that excessive folate supplementation provided no additional benefit (350). Similarly, cell models have shown that folic acid deficiency increases sensitivity to radiation-induced chromosome breakage from ionizing radiation (352). Antioxidant properties of folate have been studied, and it was found to efficiently scavenge a diverse array of reactive oxygen species (354). Cell models also show the ability of folate to reduce iron toxicity in cases of iron overload by oxidizing free or chelated iron (354). Folate status during exploration missions may be even more important because of known increases in iron storage during long-duration spaceflight (31) and exposure to ionizing radiation.

### **4. Gaps**

Ongoing evaluation of one-carbon metabolism pathways, vitamins, and polymorphisms are required for better understanding of the role of vitamin B<sub>12</sub> in astronaut vision issues. Folate levels in the space food system need to be determined. If the diet does in fact provide 400 µg/d, further research should be conducted to understand the stability of folate following radiation exposure.

## **F. Thiamin**

### **1. Background**

Thiamin functions as a coenzyme in the metabolism of carbohydrates and branched-chain amino acids. The coenzyme form of thiamin, thiamin pyrophosphate (TPP), functions in the decarboxylation of pyruvate and  $\alpha$ -ketoglutarate. Without these decarboxylations, synthesis of both adenosine triphosphate (ATP) and acetyl-CoA would be inhibited. TPP also functions as part of a major enzyme involved in the hexose monophosphate shunt, the pathway by which 6-carbon sugars are converted to pentoses and NADPH. Thiamin pyrophosphate is also believed to be involved in nerve conduction and nerve membrane function, although its role is not completely clear (345).

About 30 mg of thiamin is stored in the human body (348). About half of the body's thiamin is stored in the skeletal muscle, with the rest being stored in the heart, liver, kidney, and brain. Thiamin in excess of tissue needs and storage capacity is excreted in the urine. The biological half-life of thiamin is in the range of 9-18 days.

Deficiency of thiamin ultimately leads to beriberi (enlarged heart, muscle weakness, anorexia, apathy, reduction in nerve impulse transmission), or even death. There are no known toxicity symptoms of excess thiamin.

## **2. Evidence**

Evaluation of erythrocyte transketolase activity, an index of thiamin status (359), before and after spaceflight did not yield any abnormal data (31).

The current documented spaceflight requirement for dietary intake of thiamin is 1.2 and 1.1  $\mu\text{mol/d}$  for men and women, respectively, which is the same as the U.S. recommended dietary allowances (RDAs) for men aged 19 and older and for women (348).

## **3. Risk**

It is well known that thiamin is highly susceptible to destruction by radiation (360-362) and processing in foods (363). It will be crucial to determine if thiamin can survive a 3-year-plus mission to deep space.

## **4. Gaps**

The thiamin levels in the space food supply and their stability need to be determined, particularly because thiamin is highly susceptible to degradation from radiation exposure.

## **G. Niacin**

### **1. Background**

The term “niacin” includes nicotinamide, nicotinic acid, and their derivatives that have the biological activity of nicotinamide (348). In its coenzyme forms, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), niacin has many different metabolic roles in the human body. The nicotinamide moiety accepts hydride ions in numerous biological redox reactions. NAD functions in respiration and as a co-dehydrogenase with enzymes involved in the oxidation of fuel molecules. NAD is converted to NADH, which transfers electrons from the Krebs cycle through the electron transport chain. NAD also acts as a donor of adenine dinucleotide phosphate-ribose for the posttranslational modification of proteins (345). The coenzyme NADP has a role in fatty acid, cholesterol, and steroid syntheses and, as a co-dehydrogenase, in the pentose phosphate pathway. Conversion of folate to its active forms also requires NADP.

Niacin is stored in the liver as NAD. This storage NAD can be converted from tryptophan, nicotinic acid, or plasma nicotinamide. Limited data show that after 80-135 days of ingesting a low-niacin diet, subjects' urinary excretion of N<sup>1</sup>-methylnicotinamide is at a level representing deficiency status (348).

Niacin in the amount of 3 g/d or more has been associated with toxic effects (348). Flushing, gastrointestinal problems, hepatotoxicity, glucose intolerance, and ophthalmic effects have all been associated with high doses of the vitamin. However, many of these toxic effects have been shown to occur only after treatment over long periods and in amounts that far exceed the recommended dietary allowance (RDA).

Deficiency of niacin leads to dermatitis, glossitis, growth retardation, and ultimately pellagra (diarrhea, dermatitis, and dementia), or even death. Toxicity from large doses of niacin can cause vasodilatory effects (flushing), gastrointestinal distress, hepatotoxicity, glucose intolerance, and blurred vision.

## **2. Evidence**

Niacin status of astronauts during and after flight has not been measured. The current documented spaceflight requirement for dietary intake of niacin is 16 mg niacin equivalent (NE)/d (97). In the U.S., the niacin requirement is 16 mg/d of NE for men aged 19 years and older, and 14 mg/d of NE for women aged 19 years and older (348). One niacin equivalent is equal to about 60 mg of the amino acid tryptophan and can be obtained from 6 g of high-quality protein (345). The RDA for niacin can be met from the actual niacin content of the diet or by conversion of tryptophan in the diet.

## **3. Risk**

Very little is known about niacin metabolism during spaceflight. One concern for exploration missions is the stability of niacin in the food system, particularly because of reports showing that the niacin content of foods decreases after exposure to 6 kGy of radiation (364).

## **4. Gaps**

Niacin content and stability in the space food supply need to be determined.

## **H. Riboflavin**

### **1. Background**

The most important biologically active forms of riboflavin are flavin mononucleotide and flavin adenine dinucleotide (FAD). These cofactors participate in a range of redox reactions in numerous metabolic pathways (365). Some of these pathways are niacin-dependent and -independent dehydrogenations, reactions with sulfur-containing compounds, hydroxylations, oxidative decarboxylations, dioxygenations, and reduction of oxygen to hydrogen peroxide. The riboflavin cofactors also play a role in the formation and function of some other vitamins, including folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub> (365).

The highest concentrations of stored riboflavin are found in the liver, kidneys, and heart (348), and almost all riboflavin in tissues is enzyme-bound, such as FAD covalently bound to succinic dehydrogenase (366). The total body stores of riboflavin are enough to meet the demands of the body for 2-6 weeks (348). Unbound flavins are labile and are rapidly hydrolyzed to release free riboflavin. Excess free riboflavin is excreted in the urine (365).

Riboflavin deficiency affects ferritin iron mobilization and iron absorption. Other symptoms of riboflavin deficiency include peripheral nerve demyelination, neurological abnormalities, and anemia.

### **2. Evidence**

Erythrocyte glutathione reductase (EGR) activation, an index of riboflavin status (359), was unchanged after flight compared with preflight (31). A 14-day exposure to increased atmospheric pressure (that is, saturation diving) did not have a statistically significant effect on EGR, but EGR activation during and after the dive tended ( $P=0.07$ ) to be lower than before the dive (358). The current documented spaceflight requirement for dietary intake of riboflavin is 1.3 mg/d. In the U.S., the recommended dietary allowance for riboflavin for men and women aged 19 years and older is 1.3 and 1.1 mg/d, respectively (348).

### **3. Risk**

Cataract incidence is higher in space travelers than the general population (271), and cataracts have also been described in riboflavin-deficient animal models (365, 367). Although no evidence exists that riboflavin status changes during spaceflight, the possibility that this nutrient could be involved in cataract formation cannot be ignored.

Riboflavin is relatively heat-stable, but it is readily degraded by light (365, 368). It does not seem to be degraded by  $\gamma$ -radiation of foods (361, 369).

### **4. Gaps**

No evidence exists that riboflavin status is altered during 4 to 6-months of spaceflight (31); however, riboflavin content in the space food supply needs to be investigated to ensure that riboflavin will not degrade during long-duration storage. Riboflavin is relatively heat-stable, but it is readily degraded by light (365, 368). It does not seem to be degraded by  $\gamma$ -irradiation of foods (361, 369).

## **I. Vitamin C**

### **1. Background**

The term “vitamin C” actually refers to two different compounds, both of which have activity against scurvy: ascorbic acid and dehydroascorbic acid. 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. Vitamin C also provides antioxidant protection by returning  $\alpha$ -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.

It has been suggested that vitamin C requirements should be greater in individuals 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 (322).

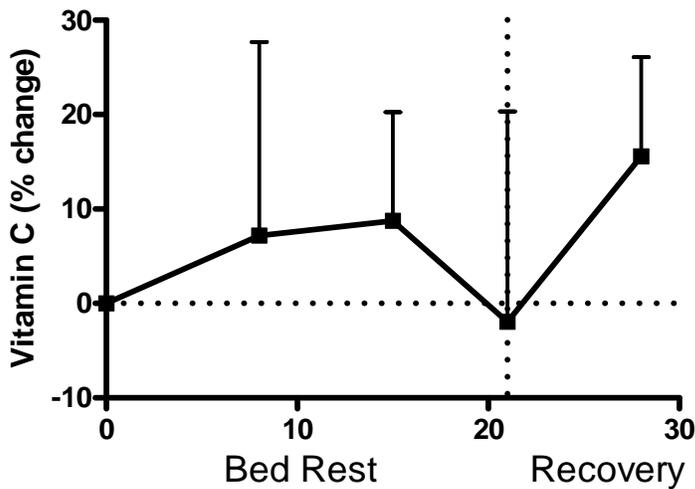
The total body pool of vitamin C varies with intake. Higher concentrations are found in the pituitary and adrenal glands, liver, spleen, heart, kidneys, lungs, pancreas, leukocytes, eye tissues and humors, and brain, while lower concentrations are found in the saliva, muscle, and plasma. Blood cell and tissue concentrations become saturated at intakes of 100-140 mg/d, and steady-state plasma vitamin C concentrations occur with intakes of 200 mg/d. Catabolic turnover varies from 10-45 mg/d, and turnover is reduced with low intakes. Maximum body pools of ascorbate are ~2 g.

Vitamin C deficiency most commonly presents as any of an array of symptoms commonly referred to as scurvy. Scurvy is seen in adults within 45-80 days of stopping vitamin C intake. Intake below the recommended dietary allowance (RDA) can cause a deficiency once the body pools fall below ~300 mg of ascorbic acid. The length of time until scurvy symptoms develop when intake is suboptimal depends on the size of the individual’s body pool of vitamin C before intake is decreased.

Deficiency of vitamin C leads to fatigue, depressed immune function, and ultimately scurvy (fatigue, muscle cramps, bruised and/or bleeding gums), or even death. Toxic amounts of vitamin C lead to gastrointestinal distress.

### **2. Evidence**

Vitamin C assessments from ISS have not been completed yet, but they will be available soon and may raise additional questions. A recent short-duration bed rest study documented no statistically significant changes in vitamin C (Figure 43), but results showed a trend toward an increase, which might be related to dietary intake or plasma volume shifts (356).



**Figure 43.** Plasma vitamin C in 7 subjects during 21 d of bed rest. Data are expressed as the percentage change (mean±SD) from before bed rest (356). The vertical dotted line marks the end of bed rest.

The stability of vitamin C has been studied in food supplies, and it is generally unstable at a neutral or alkaline pH, or in high-oxygen environments (370). Vitamin C is also unstable when exposed to light or heat (370), as well as in irradiated foods (371, 372). Salem (372) 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 are also evident in commercial spices, such as basil, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage (373). Exposure of these spices to  $\gamma$ -rays for >3 months resulted in a marked increase in quinone radicals.

The current documented spaceflight requirement for dietary intake of vitamin C is 90 mg/d. In the U.S., the RDAs for vitamin C for men and women aged 19 years and older are 90 and 75 mg/d, respectively. They are set by assuming a coefficient of variation of 10% because information about the standard deviation is unavailable. The RDA is defined as equal to the estimated average requirement (EAR) plus twice the coefficient of variation to cover the needs of at least 98% of the population (322). Because of the increased level of stress predicted for orbiting crews, the requirement for vitamin C in space crews was initially defined as 100 mg/d for men and women (4, 5), at a time when the U.S. RDA was 60 mg/d.

### 3. Risk

One concern for spaceflight 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 (up to 5 y).

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 (374, 375). Just as important to consider, however, is the possibility that vitamin C could induce DNA damage. Cai and colleagues (375) 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 (376). Thus, vitamin C can serve as both a pro-oxidant and an antioxidant.

#### **4. Gaps**

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.

### **J. Vitamin B<sub>6</sub>**

#### **1. Background**

Vitamin B<sub>6</sub> comprises a group of three compounds and their 5'-phosphates (P): pyridoxal (PL) and PLP, pyridoxine (PN) and PNP, and pyridoxamine (PM) and PMP. These vitamers of B<sub>6</sub> serve as coenzymes in many transamination reactions by forming a Schiff's base with the ε-amino group of lysine and the carbonyl group of PLP (348, 377). They can also function in decarboxylation reactions, such as the formation of γ-aminobutyric acid from glutamate and serotonin from 5-hydroxytryptophan, and they function in trans- and desulhydration, where cysteine is synthesized from methionine and pyruvate is generated from cysteine. The vitamers also function in cleavage reactions, racemization of D- and L-amino acids, synthesis of multiple compounds, glycogen catabolism (where vitamin B<sub>6</sub> is required for the activity of glycogen phosphorylase), and steroid hormone action (where the vitamers decrease the actions of steroids) (345).

About 80% of vitamin B<sub>6</sub> is stored in muscle tissue and 10% is stored in the liver, with the rest being stored in the blood plasma pool. Data from studies have shown that total body stores are about 1,000 μmol or 167 mg (348). Overall body half-lives of the vitamers of vitamin B<sub>6</sub> are about 25 days (348, 378).

Deficiency of vitamin B<sub>6</sub> leads to dermatitis, microcytic anemia, convulsions, altered mental status, hyperhomocysteinemia, or even death. Toxic levels of vitamin B<sub>6</sub> lead to sensory neuropathy or even death.

## **2. Evidence**

No change occurred in the activation of red blood cell transaminase of astronauts on 4- to 6-month spaceflights (31). 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 (379). As vitamin B<sub>6</sub> is stored mainly in muscle tissue (380), a decrease in muscle cross-sectional area could reduce the amount of the vitamin that is stored. Increased excretion of 4-pyridoxic acid during bed rest, a finding observed in short- (356) and long-duration bed rest studies (381), likely reflects this loss of muscle stores of vitamin B<sub>6</sub>.

There is no evidence that vitamin B<sub>6</sub> status changes during long-duration spaceflight; 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 (349). Although increased levels of these metabolites do not point to a vitamin B<sub>6</sub> deficiency, they do suggest there may be a perturbation in the 1-carbon metabolism pathway, which is dependent on folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>.

The current documented spaceflight requirement for dietary intake of vitamin B<sub>6</sub> is 1.7 mg/d. In the U.S., the vitamin B<sub>6</sub> requirement for all adults over age 19 years is 1.3 mg/d (348).

## **3. Risk**

Given the changes observed in vitamin B<sub>6</sub> metabolism during bed rest, vitamin B<sub>6</sub> status during and after long-duration spaceflight warrants further attention. A deficiency in vitamin B<sub>6</sub> causes a decrease in the synthesis of serotonin and catecholamines, which has been shown to be associated with depression (382). Excess vitamin B<sub>6</sub> can lead to neuropathy (383-385).

## **4. Gaps**

Given the changes observed in vitamin B<sub>6</sub> metabolism during bed rest and with muscle loss, further attention is warranted. Vitamin B<sub>6</sub> levels and stability in the space food supply need to be determined, along with an assessment of stability in an elevated radiation field.

## **K. Vitamin B<sub>12</sub>**

### **1. Background**

Vitamin B<sub>12</sub> functions in many enzymatic reactions, and deficiencies result in anemia, as well as neurological disorders. Vitamin B<sub>12</sub> 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 β-leucine. Vitamin B<sub>12</sub> deficiency may cause the accumulation of folate in the serum because of a reduction in B<sub>12</sub>-dependent methyltransferase, also known as the methyl-folate trap (386). Vitamin B<sub>12</sub> also functions in the synthesis of choline, which can be converted to the neurotransmitter acetylcholine.

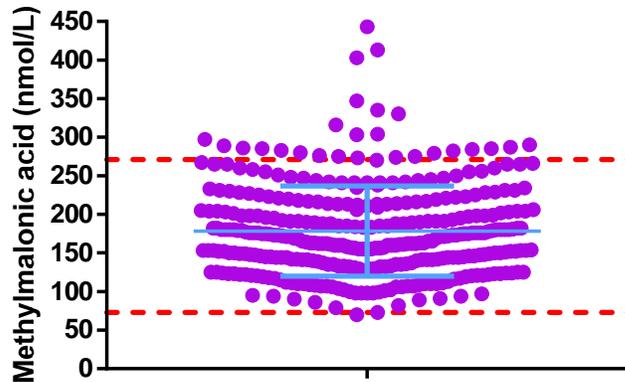
Unlike other water-soluble vitamins, vitamin B<sub>12</sub> can be stored in the body for years. It is stored predominantly in the liver, but smaller amounts can be found in the muscles, kidneys, bones, heart, brain, and spleen. About 2 to 5 mg of vitamin B<sub>12</sub> is stored in the body (348). The size of B<sub>12</sub> 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<sub>12</sub> in humans is 350 to 400 days (348).

No evidence of toxicity has been found with vitamin B<sub>12</sub> supplementation in amounts greater than the recommended dietary allowance (348), and no adverse effects are reported to be caused by an excess of vitamin B<sub>12</sub>. 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<sub>12</sub> deficiency include a decrease in gastric acidity, the presence of atrophic gastritis, and uncontrolled growth of bacteria accompanied by malabsorption of food-bound B<sub>12</sub> (387). Deficiency of vitamin B<sub>12</sub> leads to pernicious anemia and demyelination of the central nervous system, and can lead to death (388).

### **2. Evidence**

Methylmalonic acid (MMA) is generally unchanged during spaceflight, suggesting that vitamin B<sub>12</sub> deficiency is not a significant issue during flight. However, blood concentrations of MMA were shown to be higher in crewmembers who experienced vision-related issues after flight than in those who did not have such issues (349), and this difference was evident before, during, and after spaceflight. Several studies support the notion that perturbations in the vitamin B<sub>12</sub> metabolic pathway can cause ophthalmic health issues such as optic neuropathy and age-related macular degeneration (389-391).

Of ISS astronauts evaluated, the occurrence of off-nominal vitamin B<sub>12</sub> values has been 13% of preflight, 13% of in-flight, and 34 % of postflight assessments. That is, these percentages of astronauts studied have had at least one MMA data point outside normal limits during the specified flight phase (Figure 44).



**Figure 44.** Plasma methylmalonic acid data collected on ISS astronauts. Each point represents an individual test point, and the horizontal bars represent mean  $\pm$  SD. Dashed lines represent normal ranges (Smith and Zwart, unpublished observations).

The current documented spaceflight requirement for dietary intake of vitamin B<sub>12</sub> is 2.4  $\mu\text{g}/\text{d}$ . This is the defined required amount of vitamin B<sub>12</sub> in the U.S. for both men and women aged 19 years and older (348).

### **3. Risk**

Alterations in vitamin B<sub>12</sub> metabolism or requirements during long-duration flights could have critical health implications for the crew, and warrant further investigation, especially with respect to vision-related issues.

### **4. Gaps**

Ongoing evaluation of one-carbon-metabolism pathways, vitamins, and polymorphisms are required for better understanding of the role of vitamin B<sub>12</sub> in astronaut vision issues. As artistic depiction of this is presented in Figure 102. Given the incidence of elevated MMA findings, and of crewmembers referred to their flight surgeons for evaluation of vitamin B<sub>12</sub> status, this role of vitamin B<sub>12</sub> warrants further study. Vitamin B<sub>12</sub> levels and stability in the space food supply should be determined.

## **L. Biotin**

### **1. Background**

Biotin is a required cofactor for pyruvate carboxylase, acetyl-CoA carboxylase isoforms 1 and 2, propionyl-CoA carboxylase, and  $\beta$ -methylcrotonyl-CoA carboxylase (392). The 5 biotin-dependent enzymes are involved in carbohydrate, fatty acid, and amino acid metabolism (392), and the primary role of biotin is to transfer CO<sub>2</sub> 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 (393).

Low-biotin diets administered to 10 healthy subjects who also consumed large amounts of avidin (an egg-white protein that binds biotin very tightly) showed signs of decreased biotin status by the third day (394). Urinary excretion of biotin and its metabolites decreased significantly and urinary excretion of 3-hydroxyisovaleric acid increased significantly after 3 days; however, these decreases were not out of the normal range until days 7 and 17, respectively. Serum biotin did not decrease significantly, and it was suggested that serum biotin is not an early and sensitive indicator of marginal biotin deficiency (395). Animal studies indicate that a biotin deficiency, marked by urinary biotin excretion, generally occurs about 2-3 weeks after beginning consumption of a biotin-free diet (396, 397).

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 (398-400). 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 (401-403).

Frank biotin deficiencies are associated with neurological and dermatological manifestations, which are likely caused by the loss of function of biotin-dependent enzymes. Seizures, hearing loss, optic atrophy, dermatitis, and aciduria (associated with elevated blood concentrations of organic acids) are common symptoms of a frank biotin deficiency. There is no evidence of toxicity of biotin at high intake levels.

### **2. Evidence**

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

The current documented requirement for dietary intake of biotin during spaceflight is 30  $\mu\text{g}/\text{d}$ . In the U.S., the dietary reference intake for biotin has been based only on adequate intake (AI) data (348). To date, no recommended dietary allowance has been reported for biotin due to lack of data and a general consensus that colonic bacteria synthesize biotin that contributes to the daily supply. Because microbial synthesis of biotin takes place in the lower part of the intestine, where nutrient absorption is limited, controversy exists regarding how much of the biotin produced by colonic bacteria is available for host metabolism. The AI for adults is extrapolated from the AI for healthy infants consuming breast milk and has been determined to be 30  $\mu\text{g}/\text{d}$  for men and women >19 years.

### **3. Risk**

Virtually nothing is known about biotin as it relates to spaceflight. Alterations in metabolism or requirements during long-duration flights, or the occurrence of drug/nutrient interactions, could have significant health implications for crews.

### **4. Gaps**

Biotin levels in the space food system need to be determined. The biotin status of astronauts during and after flight, as well as the fact that gastrointestinal changes during spaceflight may lead to changes in the microbial synthesis of biotin, warrant further study. Furthermore, the interaction of biotin with some pharmacological agents (such as phenobarbital) included in the medical kit supplied to astronauts during spaceflight has been shown to yield biotin deficiencies in other populations (399).

## **M. Pantothenic Acid**

### **1. Background**

The primary function of pantothenic acid lies in its role as a precursor of coenzyme A (CoA) and as a component of acyl carrier protein (ACP). Pantothenic acid, in the form of CoA and ACP, is required for numerous lipid, carbohydrate, and protein metabolic reactions. CoA is necessary for acetyl and acyl transfer reactions associated with catabolism, and it acts as a precursor to ACP. ACP is a coenzyme in the fatty acid synthase complex.

Free pantothenic acid is found in various parts of the body: 10-15  $\mu\text{mol/L}$  in the liver,  $\sim 100$   $\mu\text{mol/L}$  in the heart, 1-5  $\mu\text{mol/L}$  in plasma, 50-100  $\mu\text{mol/L}$  as CoA, and 10  $\mu\text{mol/L}$  as ACP. About 70-90% of CoA is found in the mitochondria. Any excess pantothenic acid is excreted in urine (404).

Because pantothenic acid is widely distributed in foods, deficiencies have been reported only in cases where semisynthetic diets or antagonists to the vitamin were used. Individuals became deficient after 63 days on a diet virtually devoid of the vitamin (405).

Because pantothenic acid is required in numerous metabolic reactions, deficiency of the vitamin can cause neurological, immunological, hematological, reproductive, and gastrointestinal dysfunctions. Specific symptoms include dermatitis, growth retardation, numbness and burning of hands and feet, impaired antibody production, headache, fatigue, insomnia, increased sensitivity to insulin, and intestinal disturbances. Pantothenic acid deficiency is rare because of its presence in a wide variety of foods of both plant and animal origin. Deficiency of the vitamin is frequently associated with multi-nutrient deficiencies, making it difficult to detect specific symptoms of pantothenic acid deficiency. There is no conclusive evidence that adverse effects occur from high intakes of pantothenic acid.

### **2. Evidence**

No data regarding pantothenic acid intake or status during or after spaceflight are currently available. The current documented requirement for dietary intake of pantothenic acid during spaceflight is 30 mg/d (97). In the U.S., the dietary reference intake for pantothenic acid has been based only on adequate intake (AI) data (348). No recommended dietary allowance has been reported for pantothenic acid. The AI for adults is based on mean intakes and is 5 mg/d for men and women  $>19$  years. No upper limit has been reported for pantothenic acid, but doses of the vitamin as high as 10-20 g/d have been well tolerated, with occasional diarrhea reported (404).

### **3. Risk**

The stability of pantothenic acid under conditions of long-term spaceflight (such as extended storage time and exposure to high-linear energy transfer radiation) will have to be determined to minimize the risk for pantothenic acid deficiency symptoms.

### **4. Gaps**

Pantothenic acid levels in the space food system should be determined, as well as their stability under conditions of long-term spaceflight conditions.

## **N. Calcium**

### **1. Background**

Calcium is critical for maintaining the body's structural and mechanical functions, and it constitutes 37% to 40% of the bone mineral hydroxyapatite in the body (406). In addition to its obvious role in the musculoskeletal system, calcium has a critical role in modulation of the function of important proteins and regulation of metabolic processes. Calcium binding is responsible for the activation of a wide range of proteins, including those involved in cell motility, blood coagulation, muscle contraction, neural transmission, glandular secretion, and cell division (407, 408). Circulating calcium levels are under tight control and are maintained within a narrow range (409).

Bone acts as the body's reservoir for calcium. Total skeletal calcium is on average 1100-1500 g, and inadequate calcium intake has significant impact on adult bone (410). About 1% of the body's calcium stores resides in the intracellular structures, cell membranes, and extracellular fluids (408).

Calcium depletion is not uncommon in many subgroups of the population. During acute starvation, urinary calcium remains constant; the largest amounts of calcium loss occur in feces, with much of the mineral loss apparently coming from bone (197). Gamble et al (198) examined blood mineral concentrations in children during acute starvation and showed that calcium levels did not change after 4 days of fasting. Studies in dogs and cats indicate that significant changes occur only when more than 35% of body mass is lost (411). Blood calcium levels after chronic semi-starvation are variable, but most studies indicate that plasma or serum calcium levels decrease (197). Controlled calcium balance studies during semi-starvation provide more variable results, with individual calcium balances ranging from positive to negative (197).

Calcium absorption may be decreased in a variety of disease states, including Crohn's disease, diabetes, chronic renal failure, and malabsorption syndromes (408). Although the daily calcium intake requirement increases with age, many of the elderly and other population groups have inadequate intakes. Assessment of calcium deficiency by clinical laboratory analyses is difficult because circulating calcium is tightly regulated over a wide range of intakes (408). Imaging techniques (such as dual-energy x-ray absorptiometry and quantitative computed tomography) that enable determination of bone mineral content may provide a good indicator of long-term calcium nutritional status.

Deficiency of calcium leads to reduced bone mass and osteoporosis. An excess of calcium leads to kidney stones, hypercalcemia, and ultimately renal insufficiency or even death. Intakes up to 2500 mg/d are considered safe under normal conditions (408).

### **2. Evidence**

As a result of skeletal unloading during flight (314, 412-421), bone mineral is lost, leading to increased urinary excretion of calcium (94, 413, 415). Bone loss is a significant health concern for long-duration spaceflight (422-424). It is estimated that the rate of bone mineral loss during spaceflight is about 0.5-1% per month (417, 425, 426). The bone loss and increased risk of renal stone formation during and after flight (181, 182) are significant.

The Skylab studies showed that during spaceflight, bone mineral was not uniformly lost from all parts of the skeleton. Loss of bone tissue was most profound in weight-bearing bones

such as the *os calcis*. Of the 3 men aboard the 59-day Skylab 3 mission, 1 lost a significant amount of *os calcis* bone mineral (-7.4%) but the other 2 did not (+2.3% and +1.4 %). Calcium excretion in the urine was 200% of the preflight value for the man who lost *os calcis* mineral and 50% of the preflight values for the other 2 men (413).

While blood calcium concentrations are tightly maintained (Figure 45, Figure 46), negative calcium balance was observed during the Skylab (39, 94, 413, 415, 427, 428) and Mir (30, 63) missions. During the 84-day Skylab 4 mission, the calcium balance was -200 mg/d (94, 429), but no significant calcium losses occurred during the 28-day Skylab 2 mission (413, 425). Increased urinary and fecal calcium excretion accounts for most of the deficit in calcium (30, 39, 63, 94, 181, 413, 415, 428). During the Skylab 4 mission, calcium losses correlated roughly with mineral losses in the *os calcis* (430) and increases in the excretion of hydroxyproline.

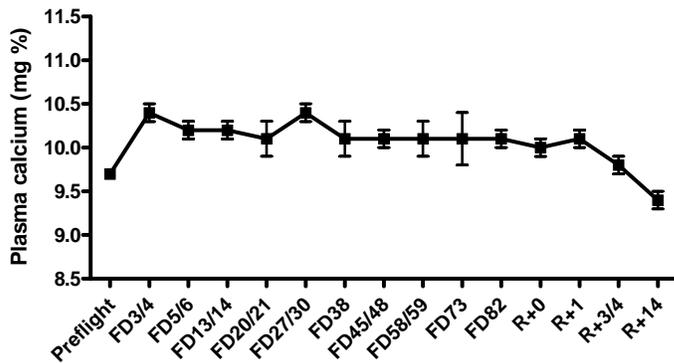


Figure 45. Plasma calcium of Skylab crewmembers (N=9) before and after flight (39).

Figure 46. Serum calcium of Shuttle crewmembers (N=2-6) during and after flight, expressed as percentage change from preflight values (208).

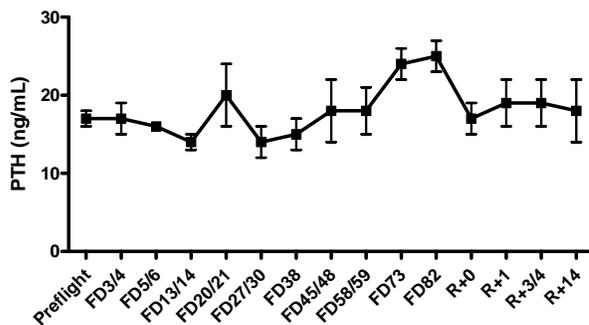
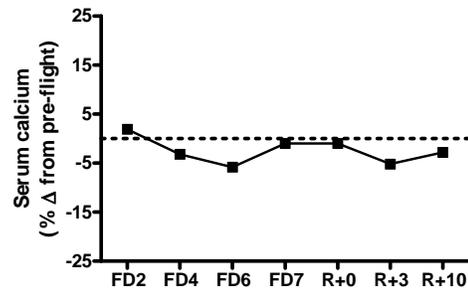
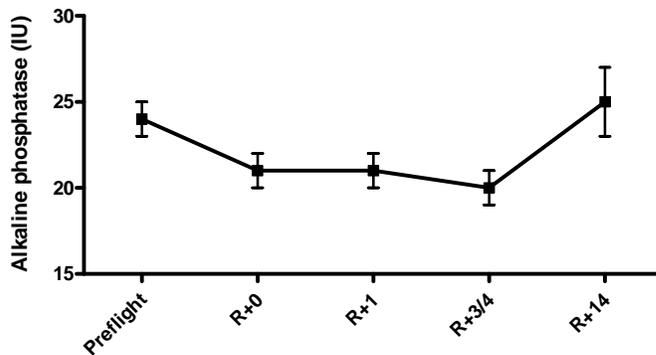


Figure 47. Plasma parathyroid hormone (PTH) concentrations of Skylab crewmembers (N=9) before and after flight (39).

Bone resorption increases during spaceflight, as shown by the concentrations of bone markers (431-433) and by the results of calcium tracer kinetic studies (30, 63). Urinary hydroxyproline was elevated 33% after 84 days of flight (94, 434). Urinary collagen crosslinks, also markers of bone resorption, were elevated >100% during spaceflight compared with preflight levels (63, 431). Calcium tracer kinetic data indicated that bone resorption increased about 50% during flight (63).

Bone formation either remains unchanged or decreases during spaceflight (30, 63, 425). As indicated by serum concentrations of bone-specific alkaline phosphatase and osteocalcin, bone formation was unchanged during Mir flights but increased 2-3 months after landing (30, 63). Trends toward decreased levels of bone formation markers were noted in two Mir studies with one subject each (432, 433). Studies, using calcium tracer techniques, of bone formation in three Mir crewmembers (30, 63) were equivocal (formation unchanged or decreased). Together, increased resorption and decreased or unchanged formation yield an overall negative calcium balance (30, 63).



**Figure 48.** Plasma total alkaline phosphatase of Skylab crewmembers (N=9) before and after flight (39).

A number of related factors likely contribute to the loss of bone mineral during weightlessness. Decreased calcium absorption has been observed among Mir astronauts (30, 63), which likely resulted from the decreased concentration of circulating 1,25-dihydroxyvitamin D that was also observed in these crewmembers (30, 63). While it is important to maintain calcium intake during flight, the lower absorption during flight suggests that this is not a viable countermeasure for weightlessness-induced bone loss, a fact proven in bed rest studies (435).

Spaceflight analog studies (such as bed rest) with humans have shown qualitative effects on bone and calcium homeostasis similar to those shown in flight studies (424), with quantitative effects generally being of smaller magnitude. Effects include loss of bone mass (436-438), decreased calcium absorption (439), increased urinary calcium and biochemical markers of resorption (415, 439-446), increased risk of renal stone formation (443, 444), and decreased serum concentrations of parathyroid hormone (PTH) (296, 441) and 1,25-dihydroxyvitamin D (296, 439, 441, 447).

Histomorphometry (438, 448) and measurement of biochemical markers of bone metabolism have shown that bone resorption increases during bed rest. Hydroxyproline excretion in bed rest subjects (439) is elevated. Collagen crosslink excretion during bed rest (431, 439) is elevated about 50% above control levels, compared with the greater than 100% increase during flight (63, 431). Excess dietary calcium will not protect against bone loss (449).

Histomorphometry data from bone biopsies also show that bone formation decreases during bed rest (438, 441, 448), but the concentrations of biochemical markers (296, 437, 439) indicate that formation is unchanged. This difference likely reflects the difference between site-specific (biopsy) and systemic (biochemical markers) indices of bone formation. After ambulation following bed rest, bone formation is generally increased (437, 439).

Bone loss and altered calcium metabolism occur in paralyzed individuals (as reviewed by (450)), and there are a number of similarities between these changes and those associated with spaceflight (451-454). The loss of bone that occurs after spinal cord injury seems to stabilize after about 25 weeks (455). Studies of bone metabolism have not been possible during space missions of this duration, and the limited postflight bone assessment does not allow determination of the rate of loss.

If the rate of bone calcium loss is constant throughout a flight (a reasonable assumption judging by collagen crosslink excretion data (30, 63, 431)), about 250 mg of bone calcium is lost per day (30, 63, 94, 456). The nature and degree of bone mineral loss over time varies between subjects (417-420, 424, 457).

Long-term follow-up data on bone recovery are far from complete (420, 458). However, if the rate of postflight recovery estimated from biochemical data is also assumed to be constant (reasonable according to ground-based (436) and flight (30, 63) data), the rate of recovery is about +100 mg/d (30, 63). By these estimates, on flights up to about 6 months, it takes 2-3 times the mission duration to recover the lost bone. For longer flights, however, the usefulness of these assumptions is questionable, as spaceflight data are not available. Although more data clearly are required to validate this hypothesis, it nevertheless has significant implications as mission durations increase.

The current documented spaceflight requirement for dietary intake of calcium during flight is 1200-2000 mg/d. In the U.S., the recommended dietary allowance is defined as 1000 mg for men and women age 19-50 years, and increases to 1200 mg/d for women 51 years and older and men 71 years and older (279).

### **3. Risk**

The ability to understand and counteract weightlessness-induced bone mineral loss will be critical for crew health and safety during and after extended-duration space station and exploration missions (459-462). Changes in the endocrine regulation of bone metabolism seem to reflect adaptation to the weightless environment. Decreases in calcium absorption and plasma levels of PTH and 1, 25-dihydroxyvitamin D are expected physiological responses to increased resorption of bone that may occur as the body adapts to an environment in which bones bear less weight. This evidence, and the lack of improvement provided by earlier dietary countermeasures, indicate that supplementation of the diet with nutrients such as calcium and vitamin D will not correct this problem (435). Adequate nutrition will, however, be a required component in the success of whatever countermeasures are identified and implemented (199, 460).

For planetary missions, the ability of a partial terrestrial *g* force (such as the 0.38*g* on Mars) to reduce bone loss, or even begin recovery, is unknown. Although no data on partial-*g* responses are available, the general consensus among investigators is that forces less than 0.5*g* are likely to be of little value.

#### **4. Gaps**

The effect of near-weightlessness on the human skeletal system is one of the greatest concerns in safely extending space missions (422, 463). Adequate intake of dietary calcium will be critical for maintaining skeletal health. Both dietary protein (amount and type) and dietary sodium affect calcium metabolism. In addition, the use of pharmacological countermeasures may have implications for calcium homeostasis. Specifically, the bisphosphonates exert their effects by inhibiting osteoclast-mediated bone resorption, lowering serum calcium in subjects who are normocalcemic or hypercalcemic (464). It is recommended that subjects receiving bisphosphonates have adequate vitamin D status before therapy and that their calcium status be monitored (465-467).

Although it is unlikely that diet is solely responsible for the bone mineral loss associated with spaceflight, even modest protective effects from a balanced diet would benefit crew health. Using diet modification as a countermeasure has several advantages, including no additional costs and no additional time required by astronauts during flight. The ratio of acid and base precursors in the diet could be an important predictor of the extent of bone loss during spaceflight and could be determined from the menu choices before flight. Maintaining a diet balanced in acid and base precursors would involve food choices and could be done with the help of a dietitian planning the menus. Furthermore, until in-flight resources for research are available, a pre- and postflight investigation of the relationship between diet and bone metabolism could provide a basis for defining optimal nutritional recommendations during recovery after spaceflight. (Small Assessment Team nutrition gap 5 is “Can one test track net bone Ca<sup>+</sup> changes?”)

## O. Phosphorus

### 1. Background

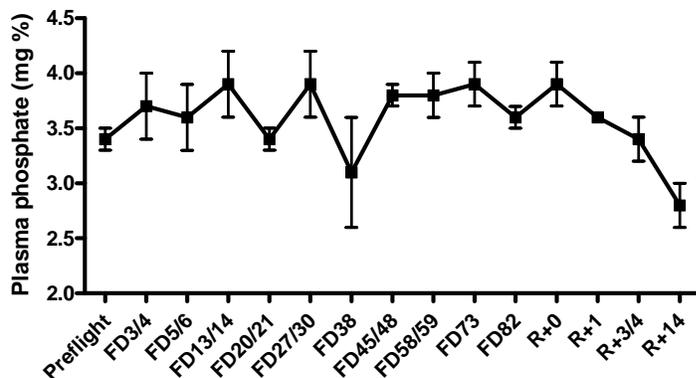
Phosphorus is an important component of cell membranes and bone. Phosphate accounts for about 60% of bone mineral (406), and most (80%) of the body's extracellular phosphorus is present in the bone as hydroxyapatite (468). Phosphorus is also a critical element of most enzymes, cellular messengers, and carbohydrate fuels.

Deficiency of phosphorus leads to hypophosphatemia, which causes cellular dysfunction and can lead to anorexia, muscle weakness, bone pain, and ultimately rickets, or even death. Osteomalacia, a defect in bone mineralization, often occurs as a result of long-term phosphorus deficiencies. Inadequate intake of phosphorus can cause the release of calcium from bone, cardiomyopathy, and a reduction in chemotactic, phagocytic, and bactericidal properties of granulocytes (468). An excess of phosphorus leads to hyperphosphatemia, ectopic calcification of the kidney, or even death. Excessive phosphorus intake has been shown to affect calcium absorption by increasing excretion of endogenous calcium in the feces (469).

Human studies show that phosphorus can be depleted by daily antacid treatment with either magnesium-aluminum hydroxide (60 mL, 4 times per day) or aluminum hydroxide (30 mL, 4 times per day) (470). Serum calcium of these subjects was elevated within 12 days of treatment, and by day 20, the phosphorus balance was negative (470). Animal studies have demonstrated that the removal of phosphate from the diet rapidly induces hypercalcemia, hypercalciuria, and hypophosphaturia. Rats fed a low-phosphate diet showed signs of deficiency after 11 days (471).

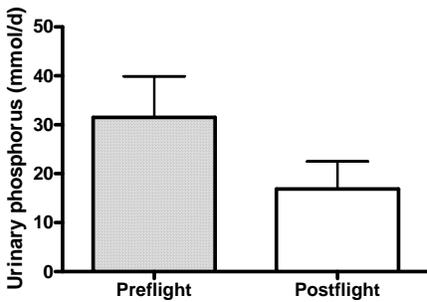
### 2. Evidence

Plasma phosphate was determined in Skylab crewmembers before, during, and after flight (Figure 49).



**Figure 49.** Plasma phosphate of Skylab crewmembers before, during, and after flight (2).

Long-duration ISS spaceflight data show that urinary phosphorus concentrations are about 45% less after landing than before launch (Figure 50) (31).



**Figure 50.** Urinary phosphorus of ISS crewmembers (N=11) before and after long-duration spaceflight. Adapted from (31).

In the U.S., the recommended dietary allowance for phosphorus (410) is 700 mg/d for men and women 19 years of age and older. The current documented spaceflight requirement for dietary intake of phosphorus during flight is 700 mg/d, and phosphorus intake should not exceed 1.5 times the calcium intake (97).

### **3. Risk**

Adequate phosphorus intake before and during flight will be critical for preserving bone quality and quantity. In addition, a dietary calcium:phosphorus ratio greater than 1.5 is known to decrease calcium absorption, which could 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 (425). Because phosphorus deficiency can cause muscle weakness and osteomalacia, maintaining adequate status of phosphorus during flight will be critical for preventing impaired performance on landing, which could limit crew capability for getting out of the spacecraft in an emergency.

### **4. Gaps**

Nominal determinations of phosphorus content of the space food system are required, as well as further investigation of the mechanism and implications of decreased phosphorus excretion after long-duration spaceflight. Small Assessment Team nutrition gap 7 is “What are the potassium, magnesium, and phosphorus changes in relation to cardiovascular issues and bone loss?”

## P. Magnesium

### 1. Background

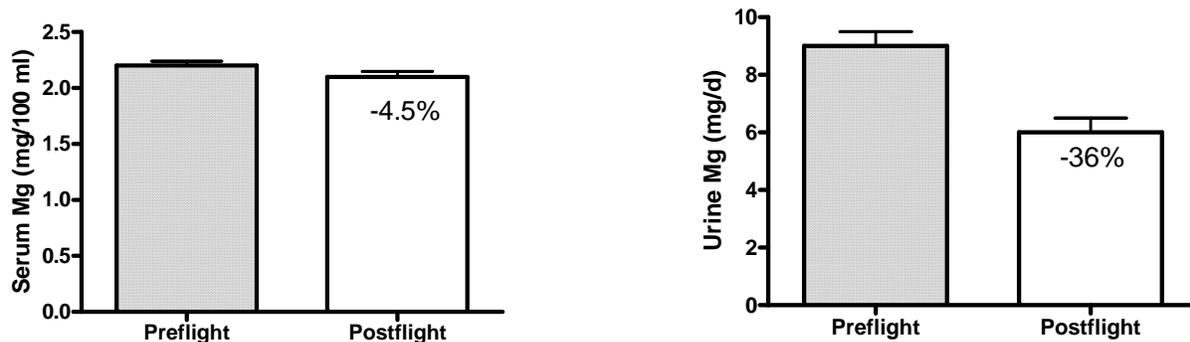
Magnesium is the fourth most abundant cation in the body, and within the cell its abundance is second only to that of potassium (472). It is required as a cofactor for over 300 enzyme systems and serves as a substrate for phosphate transfer reactions in all cells. More than half of the body's magnesium is contained in bone, about 30% in muscle, and the remainder mostly in soft tissue (473).

Few studies have addressed experimental magnesium depletion in humans. Consuming a diet containing 10 mg/d for 110 days led to a steady decline in plasma magnesium to levels 10-30% of control values, and urinary magnesium levels were negligible (< 1 mEq/d) within 7 days (473). Abnormal neuromuscular signs occurred in 5 of 7 subjects after 25-110 days of magnesium deficiency (473). Deficiency of magnesium leads to neuromuscular hyperexcitability, seizures, cardiac complications, or even death (410). Adequate intake of magnesium is necessary to prevent hypocalcemia, resistance to vitamin D, and resistance to parathyroid hormone.

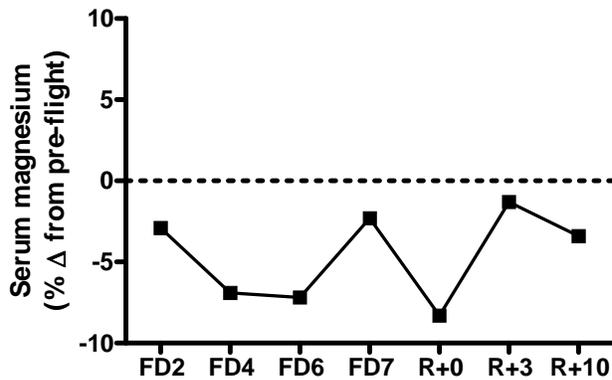
No evidence has been reported of adverse effects associated with toxicity from naturally occurring sources of magnesium, but large doses may cause gastrointestinal distress. Excessive intake from supplements has been shown to impair calcium absorption (473).

### 2. Evidence

Apollo serum and urinary magnesium levels are shown in Figure 51 (104), and in-flight and postflight Shuttle data are shown in Figure 52.

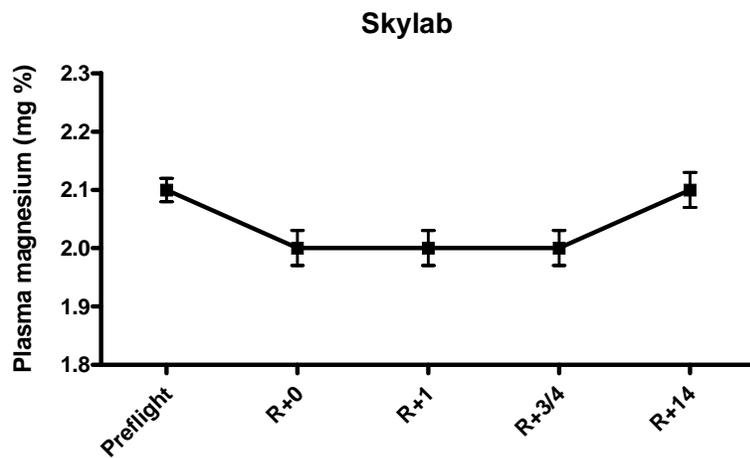


**Figure 51.** Serum (N=32) and urinary (N=23) magnesium levels in Apollo crewmembers. Numbers in bars represent the percentage change from preflight values. Adapted from (104).



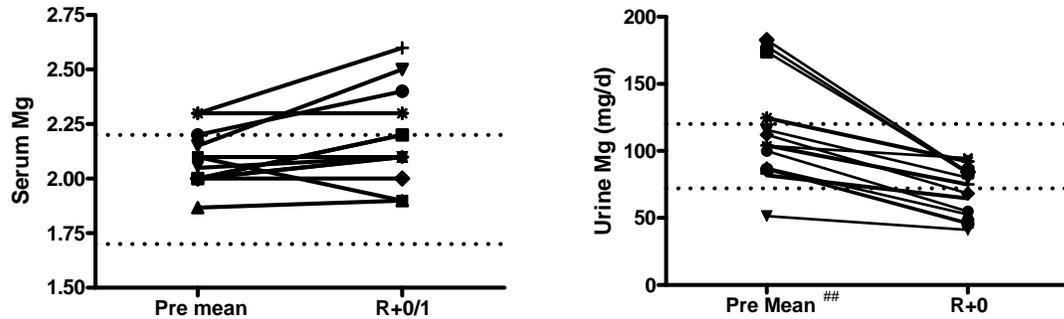
**Figure 52.** Serum magnesium levels of Shuttle crewmembers (N=2-6) during and after flight, expressed as a percentage change from preflight (208).

Crewmembers on Skylab flights had lower plasma magnesium levels at landing (Figure 53).



**Figure 53.** Plasma magnesium levels of Skylab crewmembers (N=9) before and 0, 1, 3-4, and 14 days after flight (2).

Several studies show that magnesium metabolism may be altered during and after long-duration spaceflight (31, 39, 474). After crewmembers had spent 4 to 6 months in space, their urinary magnesium was about 45% less than it was before flight (Figure 54) (31). The causes and implications of this are being evaluated in ongoing ground-based and flight studies.



**Figure 54.** Serum (left panel) and urinary (right panel) magnesium before and after 4- to 6-month spaceflights on the International Space Station. Each line represents one crewmember. The “Pre Mean” point on each line is the average of data collected about 6 months and 6 weeks before launch. R+0 = Recovery plus zero days, i.e., landing day. These samples are typically collected 2-8 hours after landing. From (1, 31).

A Russian report on the impact of spaceflight on the magnesium content of bones documented 32% lower concentrations in the compact layer of the femoral epiphysis and diaphysis, vertebral body, and sternum compared with non-flight controls (475). These changes were reported as appearing “with a high degree of certainty.” No changes were observed in the calcaneus. (Note: this study reported on the autopsy results following the tragic end of the 24-day Salyut 1 mission, compared with controls.)

The current documented requirement for dietary intake of magnesium during spaceflight is 420 and 320 mg/d for men and women, respectively (97). In the U.S., the recommended dietary allowance (RDA) for magnesium (410) is 400 mg/d for men aged 19-30 years and 420 mg/d for men aged 31-70 y. The RDA is 310 mg/d for women aged 19-30 years and 320 mg/d for those aged 31-70 y.

### 3. Risk

Adequate magnesium intake before and during flight will be critical to reducing the potential for altered magnesium status and to help preserve bone quality and quantity. Maintaining adequate magnesium status during flight will be critical for maintaining musculoskeletal structure and function and, thus, for preventing impaired performance on landing, which may limit crew capability of getting out of the spacecraft in an emergency.

### 4. Gaps

Nominal determinations of magnesium content of the space food system are required. The significant decrease in urinary magnesium excretion after 4- to 6-month spaceflights (31) also warrants further investigation.

## **Q. Iron**

### **1. Background**

Iron is an essential element involved in oxygen transport, oxidative phosphorylation in carbohydrate and lipid metabolism, and electron transport in cytochromes and cytochrome oxidase (476, 477). 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 (478). Ferritin is exponentially correlated with storage iron, as determined by quantitative phlebotomy in patients with iron overload (479).

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 (480-482). Toxic amounts 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 (483). Adaptation of iron metabolism in humans typically allows the maintenance of normal body iron concentrations in spite of disparate physiological requirements and dietary supply (484). Body iron, about 4 g in the adult human, is determined by physiological iron demands, dietary supply, and adaptation (476, 484, 485). 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 (476). Conversely, meat, fish, poultry, and ascorbic acid will enhance the bioavailability of non-heme iron.

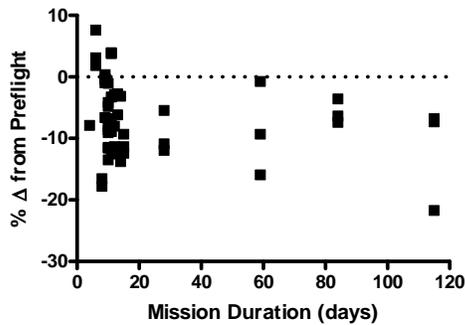
Iron is known to be involved in immune system function—specifically, adaptive and innate immune response—and both iron overload and iron deficiency affect immune function. As reviewed by Dao and Meydani, iron overload can affect susceptibility to infection (486). On the other hand, iron deficiency affects the function of certain immune cells, including neutrophils and natural killer cells, and production of cytokines (486). 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 the risk of infection, suggesting that the supplemented iron provides an environment for pathogens to thrive (487). Others have shown that iron deficiency can help protect against some types of infections (488).

## **2. Evidence**

Evidence from short- (weeks) and long-duration (months) space missions shows that red blood cell (RBC) mass decreases during flight because of neocytolysis (489, 490). An early hypothesis for the cause of decreased RBC mass was that RBC synthesis in space was understimulated relative to synthesis on the ground (491). 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 spaceflight, but it returns to preflight levels later and iron turnover is unchanged during flight (490, 492), indicating that synthesis of RBCs and hemoglobin is unchanged. A consequence of the decreased RBC 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 spaceflights (31, 474, 493). 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  $20 \pm 6$  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 spaceflight requirement for iron is 8-10 mg/d for both men and women (2, 97), and the current U.S. Dietary Reference Intake (DRI) for men is 8 mg/d and the DRI for women is 10 mg/d (277). The tolerable upper intake limit for iron as defined by the Institute of Medicine is 45 mg/d (277).

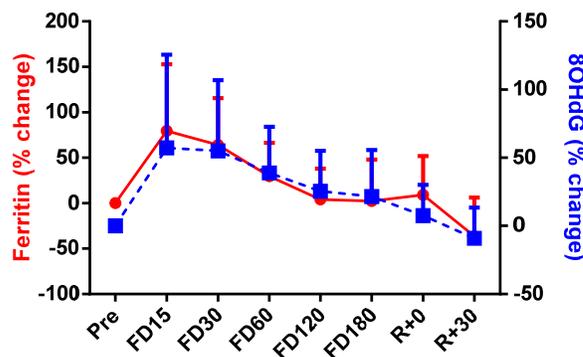
Indices of iron metabolism and erythropoiesis return toward normal relatively quickly (days) after landing, although the replenishment of RBC mass may take several weeks. The repletion of RBCs usually occurs after the disproportionate return of plasma volume, so that a dilutional “anemia” often occurs after flight (494). 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 (494).

Although the in-flight decrease in RBC mass is substantial (Figure 55), 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 (43, 495-497). 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 spaceflight. The decrease in RBC mass has no documented functional consequences.



**Figure 55.** Red blood cell mass (mL/kg body mass) after spaceflight. Each point represents one crewmember. Data are expressed as the percentage change from preflight values. From (493).

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 (498). 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 (498). At several time points, the transferrin index exceeded 1  $\mu\text{mol iron}/\mu\text{mol transferrin}$ , which provides evidence that iron overload occurred (499). 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 spaceflight (498). The change in ferritin over the course of a 6-month mission is presented below (Figure 56), and is very similar to the change in urinary 8-hydroxy-2'-deoxyguanosine (8OHdG, a marker for oxidative damage) during spaceflight). 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 (500-502).



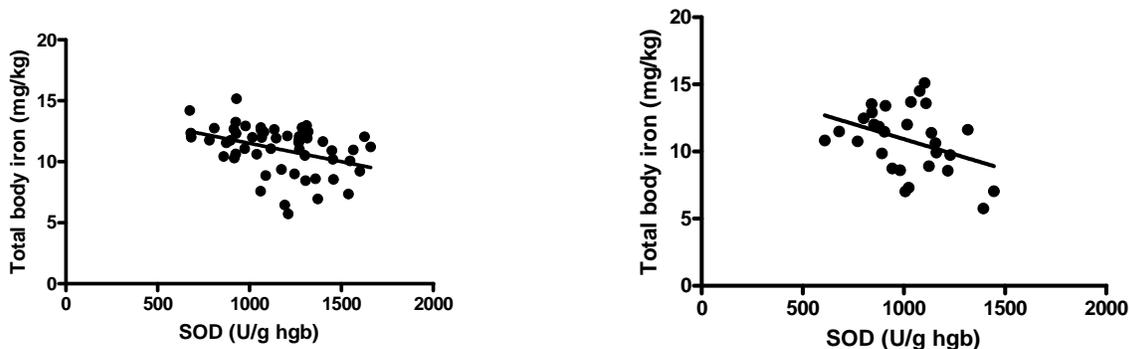
**Figure 56.** The percentage change from preflight in serum ferritin (red circles, solid line) and urinary 8OHdG (blue squares, dashed line) before, during, and after long-duration spaceflight (N=23). “Pre” was determined from the mean of preflight data points (3 for ferritin, 4 for 8OHdG), and percentage change was calculated from that average. Adapted from (498).

Bed rest studies have not proven to be consistently reliable models for the hematological changes of spaceflight. Early bed rest studies showed a decrease in RBC mass during bed rest, but erythropoietin was unchanged and hematocrit increased (503), 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 (356, 357), 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 spaceflight (decreased red cell mass, increased iron storage) (504). Exogenous erythropoietin prevented the changes (504), suggesting that it is involved in the regulating mechanism, as it may be in the initial change in spaceflight.

The NASA Extreme Environment Mission Operations (NEEMO) undersea environment provides an excellent spaceflight analog for changes in iron status, specifically with respect to the environment in the NEEMO habitat (358). Probably because of the increased air (including oxygen) pressure in the habitat, and greater oxygen availability, body iron stores are elevated during the NEEMO 10- to 14-day saturation dives (31, 493). As discussed above, excess iron can act as an oxidant and cause tissue damage (505, 506). In several NEEMO missions, we found that total body iron was related to markers of oxidative damage (Figure 57) (507).



**Figure 57.** Relationship between iron stores and oxidative damage in three NEEMO missions, where crewmembers were exposed to saturation dive conditions for 10-12 days. The panel on the left presents data from 12 crewmembers on the NEEMO 12 and 13 missions (507), and the panel on the right presents data from 6 crewmembers on the NEEMO 5 mission (358). The regression line in both graphs is significant ( $P < 0.05$ ). Adapted from (2, 507).

Serum ferritin is routinely increased during NEEMO dives, and evidence for oxidative damage and stress is also observed (358). On a recent NEEMO mission, RBC folate was decreased during the dive, and plasma folate status was inversely correlated with serum ferritin (355). Decreased superoxide dismutase activity and peripheral blood mononuclear cell poly(ADP-ribose) were also evident during the dive, indicating that a DNA repair response occurred (355). Exposure to higher oxygen pressures also increases crewmembers' risk for oxidative damage to DNA, proteins, and lipids (508-511).

The current documented spaceflight requirement for dietary intake of iron is 8-10 mg/d for men and women. In the U.S., the recommended dietary allowance (RDA) is 8 mg/d for men aged 19-70 years and 18 mg/d for women aged 19-50 y, dropping to 8 mg/d in women over 50. Historical spaceflight iron requirements for missions of 30-120 days were to be less than 10

mg/d (4, 5), matching the RDA at the time. Regardless of whether the requirement was less than 8 mg/d or 10 mg/d, the food system was unable to support this requirement, and intakes have often been much higher (intakes as high as 20-25 mg iron/d have been observed). This high intake is worthy of concern because of the potential for elevated tissue iron to cause deleterious effects.

### **3. Risk**

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 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 in healthy subjects (512). This finding supports what we have observed during spaceflight (498). Other risks with iron overload are retinal degeneration and cataract risk (513). 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 (514). Furthermore, the formation of free radicals subsequent to elevation of iron stores has also been linked on Earth to cardiovascular disease and cancer. Although aspects of some of the evidence supporting this thesis contradict each other (515, 516), a correlation between coronary heart disease and iron status has been described in a number of recent studies (517-519), and an association between increased incidence of myocardial infarction and increased iron stores (as measured by serum ferritin) has been observed (519, 520). In a prospective Finnish study, increased risk of all cancer types combined and colorectal cancer in particular was associated with high iron stores (521). The relationship between iron, lipids, and cancer has also been documented in the Framingham study (522). 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 (523). These data suggest that the alterations in erythropoiesis and iron metabolism that occur in microgravity could cause significant changes affecting crew health.

### **4. Gaps**

Better characterization of iron metabolism during spaceflight 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 spaceflight, complicated by increased radiation levels. It is known that bacterial virulence increases upon exposure to microgravity (524), and ground studies also show that increased iron status can increase risk for infection (525). 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 flight. Furthermore, iron absorption has yet to be determined during flight.

## R. Copper

### 1. Background

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 (526). Deficiencies in copper have implications for bone health, the nervous system, immune function, the cardiovascular system, and lipid metabolism (526). The involvement of copper in bone health is specifically related to lysyl oxidase function and collagen synthesis (267, 526).

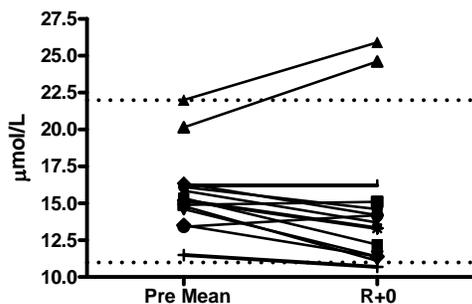
Copper is not usually stored in tissues, but liver, brain, and kidney typically contain the largest amounts per unit tissue mass (526). Total body copper is about 50 to 120 mg (0.79-1.9 mmol) (527). Copper transport and regulation involve the blood protein ceruloplasmin.

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 (528). Six patients fed (through the gastrointestinal tract) a diet containing 15 µg copper/100 kcal for 12 to 66 months (529) developed a copper deficiency.

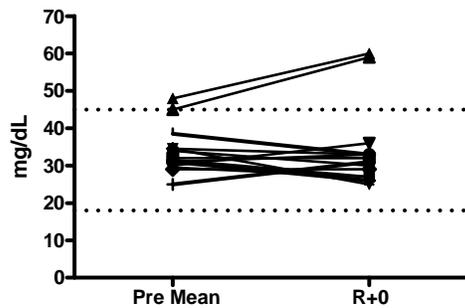
When copper deficiency occurs it leads to normocytic, hypochromic anemia; decreased production of leukocytes and neutrophils; and defects in connective tissue (specifically in collagen synthesis) that can lead to vascular and skeletal problems and central nervous system dysfunction, or even death (528). Heartbeat irregularities have also been reported in cases of copper deficiency (530). 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 (529). Toxic concentrations of copper lead to oxidative damage, gastrointestinal distress, liver damage, or even death (526).

### 2. Evidence

Serum copper (Figure 58) and ceruloplasmin (Figure 59) of ISS crews have been determined as part of the medical requirement to assess nutritional status in long-duration crewmembers, and no significant changes were noted after flight (31). In-flight determinations of copper status have not yet been reported.



**Figure 58.** Serum copper before and after 4- to 6-month missions on the International Space Station. Each line represents one crewmember. The “Pre Mean” point for each line is the average of data collected about 6 months and 6 weeks before launch. R+0 = Recovery plus zero days, i.e., landing day. These samples are typically collected 2-8 hours after landing. From (1, 31).



**Figure 59.** Serum ceruloplasmin before and after 4- to 6-month missions on the International Space Station. Each line represents one crewmember. The “Pre Mean” point on each line is the average of data collected about 6 months and 6 weeks before launch. R+0 = Recovery plus zero days, i.e., landing day. These samples are typically collected 2-8 hours after landing. From (1, 31).

One Russian report on the effect of spaceflight on copper content of bones (475) 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 reambulation it increased (531). During and after 3 weeks of bed rest, serum copper and ceruloplasmin were unchanged (356). After 90 days of bed rest, serum copper was slightly elevated, but the change was statistically significant (357). In 60- and 90-day bed rest studies, ceruloplasmin was unchanged (357).

The current documented spaceflight requirement for dietary intake of copper is 0.5-9 mg/d (97). In the U.S., the recommended dietary allowance for copper in men and women aged 19-70 years is 0.9 mg/d (277).

### **3. Risk**

Changes in copper status could contribute to the effects of spaceflight on bone, red blood cells (RBCs), and iron status. The changes in bone during spaceflight, described in this volume, could be exacerbated by copper deficiency and impaired collagen synthesis. Anemia of spaceflight is manifested as a reduction in circulating RBC mass with elevations in serum ferritin and iron concentrations (43, 490). Since copper is required for iron mobilization and absorption, alterations in copper status may affect iron and RBC changes during flight.

Appropriate amounts of certain nutrients, copper in particular (532), are vital for maintaining normal immune function. The immune system seems to be altered during spaceflight (102, 533-536), 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 (534, 537).

### **4. Gaps**

No information about copper absorption and metabolism during spaceflight 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.

## **S. Manganese**

### **1. Background**

Manganese can function as an enzyme activator and as part of metalloenzymes. It becomes involved in activating enzyme-catalyzed reactions by causing conformational changes in the enzyme that it binds to. Manganese can also bind directly to the substrate.

All transferases, including kinases, hydrolases, oxido-reductases, ligases, and lyases, can be activated by manganese. However, in the presence of a manganese deficiency, these enzymes can be activated by other divalent cations. Activating these enzymes gives manganese a role in the formation of components of connective tissue, urea formation, arginase activity, gluconeogenesis, the prevention of lipid peroxidation by superoxide radicals in the mitochondria, and the conversion of pyruvate to oxaloacetate in the tricarboxylic acid cycle. Studies are currently being conducted to look at the role that manganese may play in second-messenger pathways in tissues and the regulation of calcium-dependent processes (345).

Only trace amounts of manganese are found in animal tissues. Humans store about 10-20 mg of the nutrient. Although it is found in most organs and tissues, the highest concentrations are located in bone and in the liver, pancreas, and kidneys (345).

Signs of a manganese deficiency in humans have not been firmly established, partly because other cations can perform the same role. In one study, when adult men were fed a purified diet with only 0.11 mg manganese/d for 39 days, all of them developed a finely scaling rash, along with decreased serum cholesterol, increased serum calcium and phosphorus, and increased alkaline phosphatase (538).

Manganese is one of the least toxic trace minerals when it is taken orally. At excessively high intakes of manganese, absorption decreases and excretion increases to protect against toxicity. Toxic levels of manganese lead to neuropathy.

Manganese and iron compete for binding sites. At low iron intakes, manganese is absorbed at a greater rate than at higher iron intakes, such that higher iron intake inhibits manganese absorption. Likewise, higher manganese intake can inhibit iron absorption.

### **2. Evidence**

One Russian report on the impact of spaceflight on manganese content of bones (475) documented generally increased regional bone manganese content (26-187%) after flight relative to controls. (Note: this study reported on the autopsy results following the tragic end of the 24-day Salyut 1 mission, compared with controls.)

The current documented spaceflight requirement for dietary intake of manganese is 2.3 and 1.8 mg/d for men and women, respectively, the same as the adequate intake of manganese for men and women aged 19 years and older (277).

### **3. Risk**

Considering manganese's function in preventing lipid peroxidation and the increase in lipid peroxidation that occurs during spaceflight, ensuring adequate manganese intake on long spaceflights is vital to preventing and/or minimizing oxidative stress.

#### **4. Gaps**

Existing knowledge of manganese metabolism seems adequate; other than the nominal determinations of manganese content of the space food system, no other specific research is required.

### **T. Fluoride**

#### **1. Background**

Fluoride in bone exists in a rapidly exchangeable pool and a slowly exchangeable pool. In the rapidly exchangeable pool, fluoride is in the hydration shell on bone crystallites, where it is exchanged isoionically or heterionically with other ions nearby (410). The slowly exchangeable pool is mobilized during the process of bone remodeling. Fluoride has also been shown to influence the function of osteoblasts, enabling new bone to be made. An increase in fluoride absorption increases the amount absorbed by hard tissue, but urinary excretion also increases.

Ninety-nine percent of fluoride is stored in mineralized tissues, predominantly in bone. Because specific signs of fluoride deficiency have not been fully elucidated for higher animals and humans, it is not possible to estimate a relative time to depletion.

Fluoride deficiency increases the development of dental caries and may reduce the integrity of skeletal tissue (345). Supplementation of fluoride (5 or 10 mg/d) in ambulatory subjects was shown to have no impact on calcium homeostasis, but it resulted in a positive fluoride balance (539).

Toxicity with fluoride supplementation is rare but can occur with fluoride intakes greater than 10 mg/d for at least 10 years (410). Toxic levels of fluoride lead to enamel and skeletal fluorosis and osteosclerosis. High doses (>40 mg/d) also result in side effects, including bone pain and gastric irritation (540, 541).

#### **2. Evidence**

No information about the effect of spaceflight on fluoride status of astronauts is available.

In the 1970s, fluoride was evaluated as a countermeasure for bone loss associated with osteoporosis and simulated spaceflight (bed rest). Although fluoride balance was positive when subjects were supplemented with 10 mg/d, there was no impact on calcium homeostasis, and both the fluoride-treated and untreated groups lost calcium during bed rest (542).

The current documented spaceflight requirement for dietary intake of fluoride is 4 and 3 mg/d for men and women, respectively, which is the same as the adequate intake of fluoride for men and women aged 19 years and older (277).

#### **3. Risk**

Considering the loss of bone mass associated with weightlessness, adequate fluoride is necessary to ensure that the bone apatite remains intact.

#### **4. Gaps**

Existing knowledge of fluoride metabolism seems adequate; other than the nominal determinations of fluoride content of the space food system, no other specific research is required.

#### **U. Zinc**

##### **1. Background**

Zinc is a component of many enzymes, which depend on it for their catalytic activity. RNA polymerases, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase are all zinc metalloenzymes. Zinc provides structural integrity to these enzymes by binding to amino acids, and it may participate directly in the reaction at the catalytic site (345).

Tissue and cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense are all functions of zinc. In tissue growth, it is involved directly with the regulation of protein synthesis. Zinc helps to regulate transcription by binding to promoter sequences of specific genes. Cell membranes require zinc for protein-to-protein interactions and membrane proteins' conformation. Zinc may also affect the activity of enzymes attached to plasma membranes. Zinc stabilizes membrane structure by maintaining phospholipids and thiol groups in their necessary reduced state. It also prevents oxidation of the membrane by occupying sites that might otherwise be occupied by pro-oxidant metals and protects against oxidation by its role in the synthesis of the protein metallothionein. Zinc is an integral part of the hormone insulin and plays a role in carbohydrate metabolism.

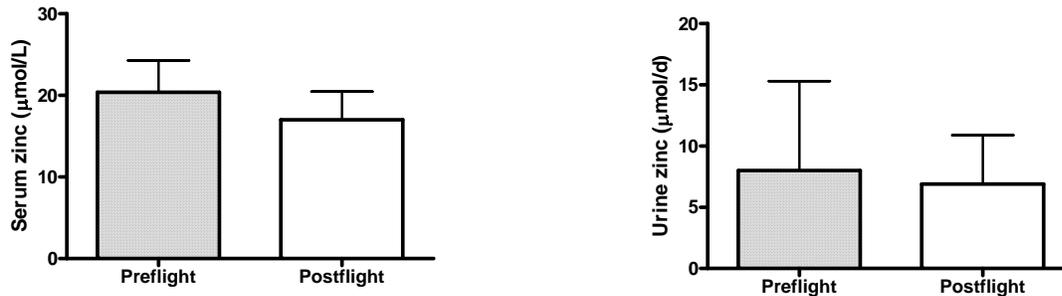
About 1.5-2.5 g of zinc is stored in the human body (277). It is found intracellularly in all organs, tissues, and body fluids but mostly in bone, liver, kidneys, muscle, and skin (345). Over 85% of zinc is found in skeletal muscle and bone (345, 543). Even when dietary zinc is suboptimal, the zinc stored in muscle, brain, lung, and heart is not released. The apatite of bones releases zinc slowly, and this release does not greatly affect the zinc supply (277). The greatest losses of zinc occur through the intestine. In men, the average daily loss of zinc from sources other than the intestine remains relatively constant at 1.27 mg/d, even when individuals consume an inadequate amount of the nutrient. For women, calculation of this value has been based on the differences between men and women in average surface area and menstruation and is 1.0 mg/d (277).

Because the stores of zinc in the body are small, inadequate intake can quickly lead to exhaustion of the zinc supply. When this happens, plasma enzymes containing zinc and metallothionein are catabolized to provide the necessary zinc (277), which action yields a decrease in enzyme activity (345). Zinc deficiency can also cause decreased glucose tolerance by decreasing the insulin response. Basal metabolic rate has been shown to be decreased in individuals who were receiving a zinc-deficient diet (544). Deficiency of zinc leads to arrested growth and development and decreased immune function.

There is currently no evidence of adverse effects associated with toxicity from naturally occurring sources of zinc. However, supplemental intake may cause suppression of the immune

response, decreased high-density lipoprotein (HDL) cholesterol, reduced copper status, or even death. Acute toxicity has been shown to produce a metallic taste, nausea, vomiting, epigastric pain, abdominal cramps, and bloody diarrhea. Long-term toxicity can cause copper deficiency because zinc and copper compete for absorption by the intestine (345).

## 2. Evidence



**Figure 60.** Serum and urinary zinc status from 11 ISS crewmembers before and after flight (2, 31).

The change in the zinc status of astronauts, as assessed by serum zinc and urinary zinc excretion, did not change after long-duration spaceflight (Figure 60, (31)).

The release of zinc from bones (due to demineralization) has been noted in bed rest studies (531, 545), and a similar increase in excretion of zinc was noted in Wistar rats flown during COSMOS 1129 (a 20-day spaceflight) (546).

The current documented spaceflight requirement for dietary intake of zinc is 11 mg/d (97), the same as the U.S. adequate intake amount for men (adequate intake is 8 mg/d for women) (277).

## 3. Risk

Many compounds exist in food that can complex with zinc and decrease its absorption. Phytates, oxalates, polyphenols, fibers, and other nutrients, including vitamins, can all inhibit zinc absorption. In view of zinc's role in metabolism, it may be necessary to provide additional zinc either in the diet or as a supplement on long-duration exploration missions.

Increases in urinary zinc with increased muscle catabolism have been noted in cases of starvation or trauma (277). The importance of this phenomenon for spaceflight has not been evaluated (nor has the release of other heavy metals [such as lead] from bone during flight, although this has been modeled and proposed as a concern (547, 548)).

## 4. Gaps

Existing knowledge of zinc metabolism seems adequate; other than the nominal determinations of zinc content of the space food system, no other specific research is required.

## V. Selenium

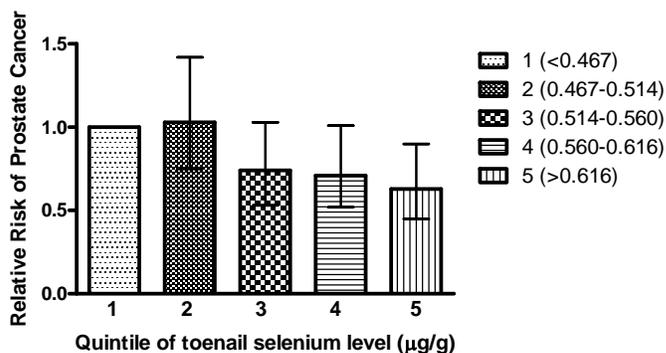
### 1. Background

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. Selenium is also a cofactor for glutathione peroxidase (GPX). GPX plays a role in the reduction of organic peroxides and hydrogen peroxide. Selenium has also been shown to be necessary for iodine metabolism.

Total body selenium stores are in the amount of about 15 mg (345). There are two selenium pools in the body: the selenium in selenomethionine and the selenium in GPX. Selenium absorption can be increased by vitamins A, C, and E and reduced glutathione, and it can be decreased by chelation and precipitation of the mineral by heavy metals, such as mercury, and phytates.

Deficiency of selenium leads to decreased selenoenzyme activity, which may lead to biochemical changes that predispose to illness or even death. Selenium deficiency has been associated with Keshan disease, characterized by cardiomyopathy and heart tissue necrosis, and with Kashin-Beck's disease, characterized by osteoarthropathy of the joints and epiphyseal-plate cartilages of the legs and arms (345). In rats, symptoms of acute selenium deficiency have been shown to appear as early as 20 days after withholding selenium. The deficiency was shown as a reduction in GPX activity, but no change in blood enzymes was seen (322).

Selenium status has been related to cancer risk (Figure 61) (549), leading to much speculation about the ability of selenium supplementation to prevent cancer.



**Figure 61.** Toenail selenium content and relative risk of prostate cancer (adapted from (549)).

Toxicity of selenium is called selenosis. Nausea, vomiting, fatigue, hair and nail brittleness and loss, changes in nail beds, interference in sulfur metabolism, and inhibition of protein synthesis have all been demonstrated to result from selenium toxicity.

### 2. Evidence

The Clinical Nutritional Assessment profile (550) has documented a significant (10%) reduction in serum selenium concentrations after flight (31); however, whether this is related to intake or metabolism is not known.

The current documented requirement for dietary intake of selenium during spaceflight is 55-400 µg/d. In the U.S., the recommended dietary allowance for men and women, aged 19 years and older, is 55 µg/d (322).

### **3. Risk**

Deficiency of selenium can lead to impaired immune function, illness, or even death. An excess of selenium can lead to nausea, vomiting, fatigue, or inhibition of protein synthesis, but it is probably not likely to occur except when selenium is consumed in large amounts in dietary supplements. Despite the relationship of selenium to cancer risk and antioxidant status, care must be taken to avoid toxicity.

### **4. Gaps**

Selenium levels in the space food system need to be determined. The potential role for selenium in protecting against oxidative stress during spaceflight should be further investigated.

## W. Iodine

### 1. Background

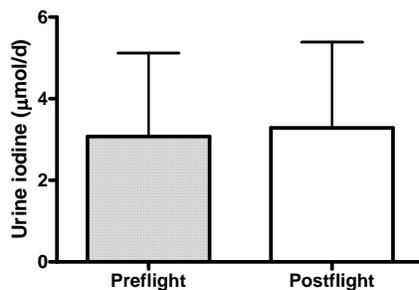
Iodine performs its main function in its ionic form, iodide, as part of the thyroid hormones T<sub>3</sub> and T<sub>4</sub>. About 15-20 mg of iodide is stored in the human body (345). The thyroid gland traps the iodide, and it is here that 70-80% of the total body iodide is stored. The rest is stored in the salivary and gastric glands, with some iodide residing in the mammary glands, ovaries, placenta, and skin.

Iodine deficiency causes iodine deficiency disorders, which include mental retardation, hypothyroidism, goiter (enlargement of the thyroid gland), cretinism, and other growth and development abnormalities, or even death. During a 4-week study in which rats were subjected to varying degrees of iodine deficiency, the most severely depleted rats showed an increase in thyroid mass after 4 days (551).

No toxic side effects have been reported when 2.0 mg of iodine per day was ingested (345). However, with prolonged intake greater than 18 mg/d, the risk of goiter increases, as does the risk of thyroid cancer (277, 410, 552). Other symptoms of iodine toxicity when intake is on the order of several grams per day include gastrointestinal distress, thyroiditis, goiter, sensitivity reactions, thyroid papillary cancer, or even death (410).

### 2. Evidence

Iodine intake on Shuttle missions was often very high, in light of the use of iodine as a bactericidal agent in the water system (553), and some related changes occurred in thyroid status of crewmembers (554-556). As a result, in the late 1990s, a system to remove iodine from water was deployed on most missions. ISS water does not use iodine, and as a result, pre- and postflight urinary iodine levels are similar (Figure 62).



**Figure 62.** Urinary iodine excretion of ISS crewmembers before and after long-duration spaceflight (31).

The current documented spaceflight requirement for the dietary intake of iodine during flight is 150 µg/d (97), the same as the U.S. recommended dietary allowance for iodine for men and women aged 19 years and older (277).

### 3. Risk

Although providing adequate amounts of dietary iodine during spaceflight is not a critical issue, there is much discussion regarding the effects of the iodine used in spacecraft water systems (where iodine is often used as a bactericide) (555). In earlier space programs, including the Space Shuttle, NASA used iodine concentrations of 2 mg/L in drinking water (553). A report from the Food and Nutrition Board of the National Academy of Sciences states that a daily iodine intake in adults ranging from 50-2000  $\mu\text{g}/\text{d}$  has no adverse effects (557). Depending on water intake, iodine intake could easily exceed 2 mg/d.

#### **4. Gaps**

Existing knowledge of iodine metabolism seems adequate. It would be prudent to know precise iodine intake levels of crewmembers (from the diet and drinking water) so that potential hazards associated with iodine excess can be avoided.

## **X. Chromium**

### **1. Background**

Chromium is thought to complex with nicotinic acid and amino acids to form glucose tolerance factor, which initiates the disulfide bridging between insulin and its receptor (345). This allows the insulin hormone to be more effective and, therefore, increases cellular glucose uptake and intracellular carbohydrate and lipid metabolism. Chromium may also play a role in pancreatic insulin secretion, internalization of insulin through decreasing membrane fluidity, and regulation of the insulin receptor. It may also increase the sensitivity of tissues to insulin by activating insulin receptor kinase.

The human body can store 4 to 6 mg of chromium. Tissues with the greatest amounts of chromium are the liver, kidney, muscle, spleen, heart, pancreas, and bone. It is possible that chromium is stored along with ferric iron because of its transport by transferrin, which can bind chromium as well as iron.

Deficiency of chromium leads to impaired glucose tolerance, or even death. Chromium deficiency may result in insulin resistance, which is characterized by hyperinsulinemia. This has been shown to be a risk factor for coronary heart disease. Several months of suboptimal chromium intake will lead to deficiency symptoms such as hyperglycemia and glycosuria (558). One study showed that 9 weeks on a low-chromium diet (5  $\mu\text{g}/1000$  kcal) was long enough to yield changes in glucose tolerance (559). Severe trauma and stress may increase the need for chromium. Stress causes the release of stress hormones, including cortisol and glucagon. These hormones alter glucose metabolism and, in effect, chromium metabolism.

Toxic levels of chromium lead to chronic renal failure, hepatic dysfunction, rhabdomyolysis (a disease of skeletal muscle), or even death.  $\text{Cr}^{6+}$  is more toxic than  $\text{Cr}^{3+}$  when ingested orally. Liver damage, skin ulcerations, dermatitis, and respiratory disease may all result from a chromium intake greater than 1,000  $\mu\text{g}/\text{d}$  (345).

## **2. Evidence**

Little or nothing is known about chromium in space travelers. Chromium deficiency may result in insulin resistance, which has also been observed after spaceflight and bed rest (108-110). Whether the insulin resistance associated with spaceflight is related to chromium is unknown. The current documented spaceflight requirement for dietary intake of chromium is 35 µg/d.

In the U.S., adequate intake of chromium is defined as 35 µg/d for men aged 19-50 years and as 30 µg/d for men aged 50 years and older. Adequate intake is defined as 25 µg/d for women aged 19-50 years and as 20 µg/d for those 50 years and older (277).

## **3. Risk**

Although it may be plausible that changes in glucose metabolism during spaceflight are partly related to chromium, given that nothing is known about chromium during spaceflight, there are no concerns about chromium at this point.

## **4. Gaps**

Existing knowledge of chromium metabolism seems adequate; other than the nominal determinations of chromium content of the space food system, no other specific research is required.

## **VII. N3.3: We need to determine changes in nutritional status due to spaceflight.**

This gap highlights the need for an evaluation of nutrition and related markers in crews during long-duration spaceflight. While one could argue that the data presented throughout this document represent those results, we describe in this section highlights from a project designed specifically to collect these data.

### **A. Background**

The medical requirement to evaluate nutritional status (Clinical Nutritional Assessment, MedB8.1) has been implemented with two Mir and all International Space Station (ISS) U.S. crewmembers to date (31), along with most USOS crews (i.e., CSA, ESA, and JAXA crewmembers as well). In 2006, a project was initiated and sought to expand the nominal medical testing in 3 ways: 1) include in-flight blood and urine collection, 2) expand nominal testing to include additional normative markers, and 3) add an R+30 session to allow evaluation of postflight nutrition and implications for rehabilitation. This expansion project was known as the Nutritional Status Assessment protocol, and given that it was defined as a Supplemental Medical Objective (SMO), was designated as SMO 016E.

Before 2006, it was not possible to assess nutritional status during flight because blood and urine could not be collected or returned during ISS missions. The findings of altered nutritional status for several nutrients after landing are worthy of concern, and we require the ability to monitor the status of these nutrients during flight to determine if there is a specific impetus or timeframe for these decrements. In addition to allowing us to monitor crew nutritional status during flight, in-flight sample collection allows better assessment of countermeasure effectiveness.

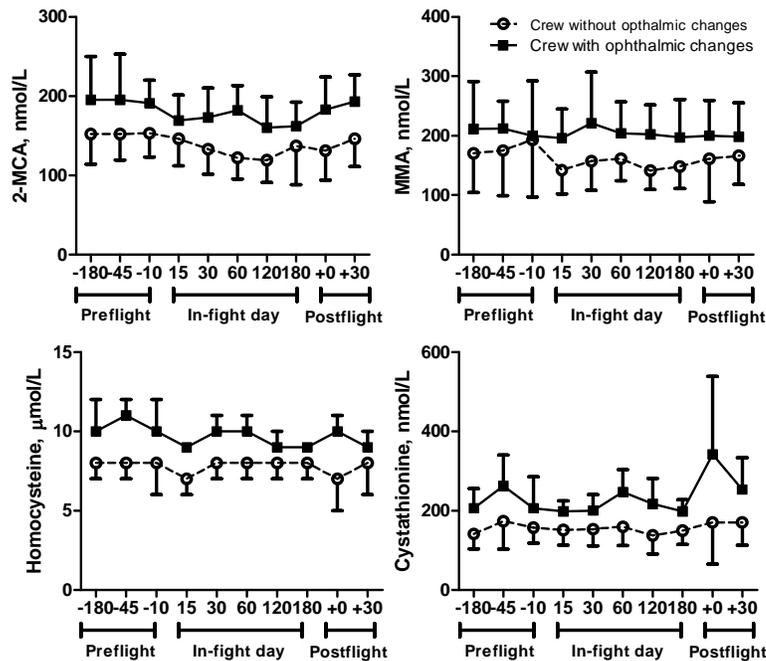
The SMO 016E protocol is also designed to expand the original Clinical Nutritional Assessment medical requirement (MR016L, later MedB8.1) to include additional normative markers for assessing crew health and countermeasure effectiveness. Additional markers of bone metabolism are measured to better monitor bone health and countermeasure efficacy. New markers of oxidative damage are measured to better assess the type of oxidative insults that occur during spaceflight. The array of nutrient assessment variables has been expanded to include better markers of vitamin status. Additionally, stress hormones and hormones that affect bone and muscle metabolism are measured. These additional assessments permit better health monitoring, and allow more accurate recommendations to be made for crew rehabilitation. Many of these variables have been included to follow the recommendations of an extramural panel that met in 2005 to define nutritional standards and requirements for spaceflight (97).

The original protocol was extended to include an additional postflight blood and urine collection (R+30). Several nutritional assessment variables are altered at landing, but it is not known if the changes are still apparent after 30 days, or whether the decrements in status on landing day require intervention for status to return to preflight levels.

## B. Highlights from Nutrition SMO

### 1. Vision

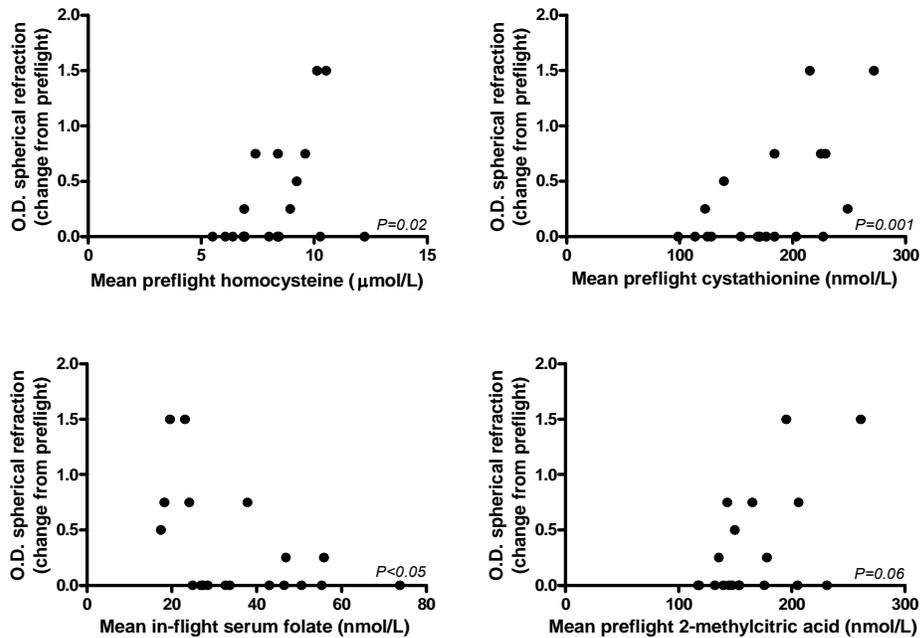
About 20% of astronauts on long-duration International Space Station (ISS) missions have developed measurable ophthalmic changes after flight (560). The Nutrition SMO 016E has provided biochemical evidence that the folate-dependent one-carbon metabolic pathway may be altered in those individuals. Serum concentrations of homocysteine, cystathionine, 2-methylcitric acid, and methylmalonic acid were significantly ( $P<0.001$ ) higher (25-45%) in astronauts with ophthalmic changes than in those without such changes (Figure 63). These differences existed before, during, and after flight. Serum folate tended to be lower ( $P=0.06$ ) in individuals with ophthalmic changes.



**Figure 63.** Serum 2-methylcitric acid (2-MCA), methylmalonic acid (MMA), homocysteine, and cystathionine before, during, and after long-duration spaceflight in crewmembers who have experienced ophthalmic changes during flight and those who have not. Figure adapted from Zwart et al (349).

Preflight serum concentrations of cystathionine and 2-methylcitric acid, and mean in-flight serum folate, were significantly ( $P<0.05$ ) correlated with changes in refraction (postflight relative to preflight, see **Figure 64**). At first, investigators thought the ophthalmic changes were caused by fluid shifts and increased intracranial pressure brought on by microgravity, but data from the Nutrition SMO 016E provided evidence for an alternative hypothesis: that individuals with this altered metabolic pathway may be predisposed to anatomic and/or physiologic changes

that render them susceptible to ophthalmologic damage during spaceflight. These data have been published (349), and a follow-on project is underway to clarify this finding.



**Figure 64.** The absolute refractive change in the right eye after flight relative to before flight was significantly associated with mean preflight homocysteine, cystathionine, and 2-methylcitric acid, and with mean in-flight serum folate. A Somers' D measure of association was used for analysis. Raw data are presented in the graphs. One crewmember with visual or intracranial pressure issues did not experience refractive changes after flight, and that crewmember had the highest preflight homocysteine and the highest in-flight serum folate, and was the only crewmember with visual or intracranial pressure issues to have reported taking multivitamin supplements during the 6-month mission. This individual also had the highest grade of disc edema of all crewmembers to date. The data from that subject are included in the graphs above (N=20 in all graphs above). Data from Zwart et al (349).

## **2. Monitoring Effects of Exercise on Bone and Body Composition**

The first ISS crews used an interim resistive exercise device (iRED). The iRED had a maximum load equivalent of 135 kg (1337 N force), an elastic force curve at the higher load ranges, and an eccentric force that was 60% to 80% of the concentric force. Early reports from ISS (419, 561) indicated that the iRED provided no greater bone protection than devices used in previous space programs (such as Mir) in which only aerobic and muscular endurance exercises were available. Studies of bone loss countermeasures on these early ISS missions were additionally confounded by the fact that the iRED was subject to several technical problems and was not usable for long periods (6, 41, 58). Although some improvements were made in the iRED, they did not overcome its inherent limitations of load quality and quantity. In late 2008, the Advanced Resistive Exercise Device (ARED) was launched to ISS. A more robust device, it has much greater resistance capability (562). It allows greater absolute loads (2675 N, 600 lb), provides a constant load throughout the range of motion, is quickly reconfigured to engage flywheels and provide variable force simulating the inertia associated with gravitational loading, and has an improved eccentric:concentric ratio of about 90%. In addition the ARED allows performance of a much greater variety of exercises. Given the site specificity of bone loss during spaceflight to weight-bearing bones (417), a highly periodized exercise program with a variety of lower-body exercises was designed to target mechanical loads to the skeletal sites displaying the greatest declines in bone mass.

With data from the Nutrition SMO and shared bone densitometry (DXA) data from operational testing, we have documented (41) that astronauts who have access to sufficient resistance exercise, coupled with adequate energy intake and vitamin D status, can return from spaceflight missions of 4 to 6 months with measured bone mass and bone mineral densities seemingly no different from baseline measures—for most skeletal regions (**Figure 65**). Although further work is needed to refine these factors (by developing optimal exercise prescriptions and optimal nutrition), the results provide the first evidence that nutrition and exercise may be able to mitigate bone loss and reduce risk for spaceflight-induced osteoporosis (41, 58).

*Risk Factor of Inadequate Nutrition*

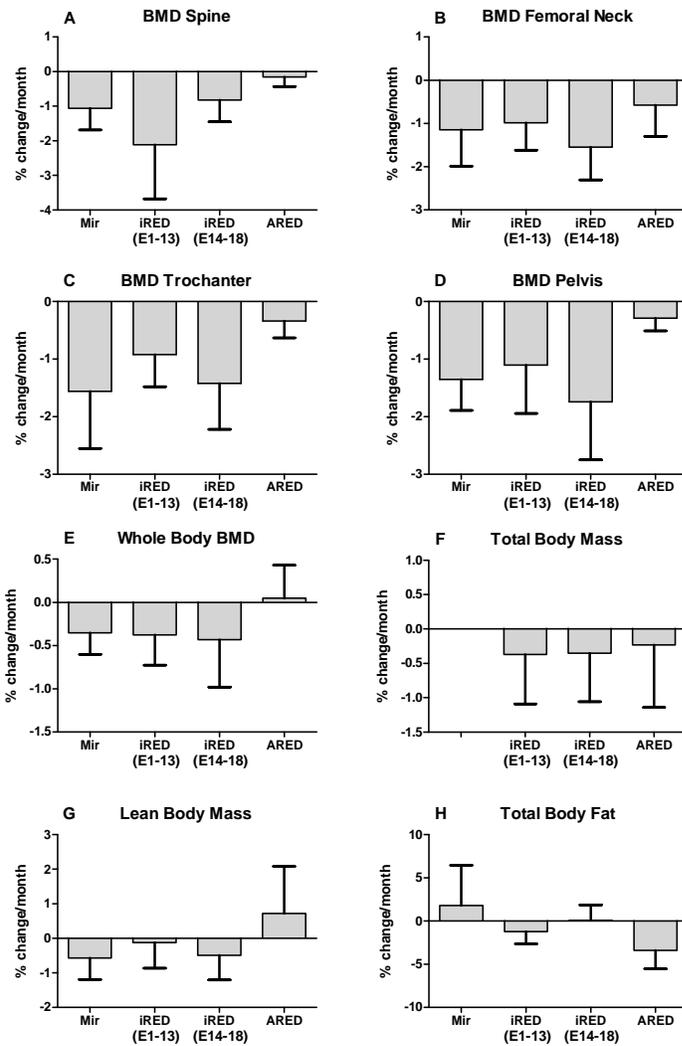


Figure 65. Bone mineral density and body composition (expressed as % change per month) in crewmembers on the Russian space station Mir, and on ISS flights with exercise on the iRED or ARED. Figure from (41).

### 3. Men and Women in Space: Effects of Exercise on Bone and Stone Risk

Sex differences in bone loss have never been carefully studied as a primary research question (563). When the paper comparing iRED and ARED exercise devices was published in 2012, the number of ISS subjects small, in part because many crewmembers participated in other countermeasure studies and were not included in that initial analysis. In 2014, an updated look at these data was published, with additional subjects included. This study confirmed the initial 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 (58), 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 (**Figure 66**) (41). 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 spaceflight that diet and exercise can maintain bone mineral density on long-duration missions.

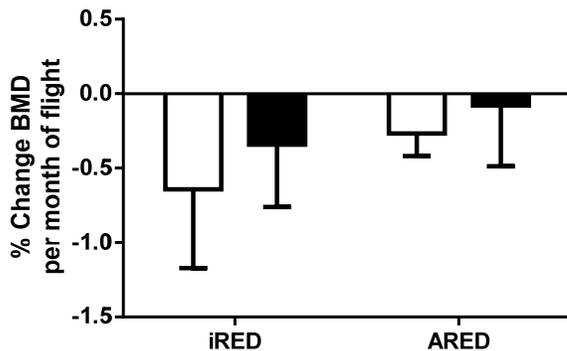


Figure 66. Bone mineral density (BMD) loss after flight in men (N=33, open bars) and women (N=9, solid bars) who used either the iRED or ARED exercise device. Data are expressed as percentage change per month of flight. Figure adapted from (58).

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 (41). In the flight study, as published, a significant ( $P<0.001$ ) increase in the bone formation marker bone-specific alkaline phosphatase occurred after landing in astronauts who had access to resistance exercise (41). In one bed rest study, heavy resistance exercise 6 days a week led to dramatic increases in bone formation markers (564). In another bed rest study with resistance exercise every other day (combined with a treadmill protocol), subjects had roughly half the bone formation response (81) of subjects in the first study (564). The exercise did not have a significant effect on serum total calcium or urinary calcium. When data from additional crewmembers who had exercised with the ARED became available, these data solidified the “trend” into statistical significance (58). The slow increase in bone formation over time during flight, despite exercise with the ARED, 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 in formation over time (41) is different from results of the first bed rest

study, in which formation markers plateaued at the first determination during bed rest (6 weeks of bed rest) (564).

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 spaceflight. 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.

#### 4. Iron Status, Oxidative Damage, and Bone

In-flight data from the Nutrition SMO indicate that iron stores increase early in flight, and then return to preflight concentrations by the end of a 6-month mission. In 23 (16 M / 7 F) crewmembers examined, ferritin increased 217% in women and 68% in men on flight day 15. Transferrin and transferrin receptors decreased later in flight, which results support the idea that mobilization of iron to storage in tissues increased. Other acute-phase proteins were not elevated during flight, suggesting that inflammation was not solely responsible for the observed increase in serum ferritin.

Although mean ferritin concentrations during flight were not outside the normal clinical range, the Nutrition SMO data showed that the increase in ferritin was associated with evidence of oxidative damage and bone resorption, an association that is supported by other studies in healthy ground-based populations (500-502). The greater the increase in ferritin during flight (or the longer it was elevated—either case would result in a larger area under the curve), the greater the decrease in bone mineral density (BMD) in the hip, trochanter, hip neck, and pelvis after long-duration spaceflight. Also supporting this result were the correlations between ferritin or transferrin index and biochemical markers of bone resorption (n-telopeptide, helical peptide) or urinary calcium during spaceflight. Several human and animal studies support these findings and show that mild iron overload (but within a normal clinical range) is associated with bone loss by a mechanism believed to be related to oxidative stress (512, 565). The change in ferritin over the course of a 6-month mission is presented below (**Figure 67**), and is very similar to the change in urinary 8OHdG (a marker for oxidative damage) during spaceflight. These findings are similar to those in animal studies by our group and others (566-568).

Although we know that consuming enough calories and exercising with the ARED mitigate changes in BMD (41), the data pertaining to iron storage and status provide evidence that the change in iron status may be another important factor to consider when defining spaceflight dietary intake requirements.

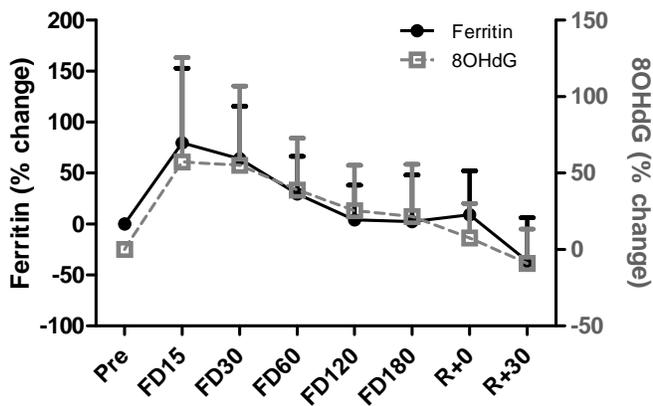


Figure 67. The percentage change in ferritin and urinary 8OHdG before, during, and after long-duration spaceflight (N=23). “Pre” was determined from the mean of preflight data points (3 for ferritin, 4 for 8OHdG), and percentage change was calculated from that average (498).

## 5. Cytokines

Assessment of plasma cytokines has been demonstrated to have clinical utility as a biomarker for various specific immunologic diseases or other disruptions in physiological homeostasis. Elevated levels of IL-6, IL-7, IL-10, and IFN $\gamma$  have been detected in the plasma of HIV-infected patients and correlate well with prognosis (569). Plasma levels of cytokines have also been found to correlate with disease presence or prognosis in rheumatoid arthritis (570), myelofibrosis (571), Sjögren syndrome (572), chronic obstructive pulmonary disease (573), and pelvic inflammatory disease (574).

It has been suggested that persistent immune dysregulation may increase specific clinical risks for astronaut crewmembers participating in exploration-class deep space missions (575). Given the paucity of cytokine data from samples obtained during long-duration spaceflight, we planned to obtain such data as part of the Nutrition SMO project. This was a cross-Discipline effort, in conjunction with the Immune Discipline, and was meant to be complementary to their studies of immune function and cytokine analysis in samples collected during relatively stressful periods of the mission. The Nutrition SMO samples were generally not collected during phases with vehicles arriving, docked, or departing, and thus samples collected for the immune function studies could provide additional information.

Findings indicated that a pattern of persistent physiological adaptations occurs during spaceflight that includes shifts in immune and hormonal regulation (Figure 68). Whether these adaptations increase crew risk for adverse medical events during spaceflight has yet to be determined. The pattern of cytokine elevations observed during spaceflight is somewhat surprising, in that within categories there are differential increases: some inflammatory cytokines or chemokines are elevated, whereas others are not. This may be explained by different cytokines having different plasma half-lives, different kinetics or magnitude of expression versus binding of cytokines by target cells, or the location and nature of the pro-inflammatory stimuli that may affect crewmembers during spaceflight. Further investigations will be required to precisely define the mechanistic causes of in-flight immune dysregulation. It is clear, however, that dysregulation of immunity during spaceflight includes the previously described alterations in cellular distribution and function (576), and newly characterized immunoregulatory alterations (**Figure 68**). Even if immunoregulatory changes are subclinical during orbital flight, clinical risk to crewmembers could be elevated for deep space missions. As studies continue to characterize in-flight immune alterations, the development of countermeasures to enable exploration missions to be conducted safely may be warranted (577).

<u>Inflammatory</u>	<u>Anti-Inflammatory</u>	<u>Adaptive/Regulatory</u>	<u>Growth Factors</u>	<u>Chemokines</u>
IL-1 $\alpha$	IL-1ra $\uparrow$	IFN $\gamma$	G-CSF	CCL2/MCP-1
IL-1 $\beta$		IL-2	GM-CSF $\uparrow$	CCL3/MIP-1 $\alpha$
TNF $\alpha$ $\uparrow$		IL-17	FGF basic	CCL4/MIP-1 $\beta$ $\uparrow$
IL-6 $\uparrow$ at landing		IL-4	Tpo $\uparrow$	CCL5/RANTES
IL-8 $\uparrow$		IL-5	VEGF $\uparrow$	CXCL5/ENA-78 $\uparrow$
		IL-10		

Figure 68. Characterization of cytokine changes during (and after) flight, based on data from (577). Arrows indicate changes observed during flight (except for IL-6, where increases were noted at landing).

## 6. Body Mass

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 (60). On ISS are two devices for measuring body mass: a Body Mass Measuring Device (BMMD), which uses spring oscillation, and a Space Linear Acceleration Mass Measuring Device (SLAMMD), which uses the physics of the equation:  $\text{force} = \text{mass} \times \text{acceleration}$ .

Maintaining body mass is a critical element of health, in space or on Earth. In 2014, we published an analysis of the body mass data from ISS, including a comparison of data collected from the two mass measuring devices (60). Crewmembers lost 2% to 5% of their body mass in the first month of flight, and subsequently maintained the lower body mass during flight (**Figure 69**) (60). Postflight measurements were lower than preflight values, supporting the notion that the loss in body mass during flight is real and not an artifact of the change in measurement device. Body mass loss can generally be explained by the subjects not consuming the number of calories recommended by the World Health Organization (60). The occurrence of a consistently lower energy intake throughout flight that was not accompanied by additional loss of body mass indicates that subjects were in energy balance during the mission. Early Space Shuttle data indicated that energy requirements are not changed on short-duration missions, but currently no data are available to address long-duration energy requirements. Energy requirements may be lower after the first ~30 days of flight, and this question is being addressed in a current ISS research protocol sponsored by the European Space Agency.

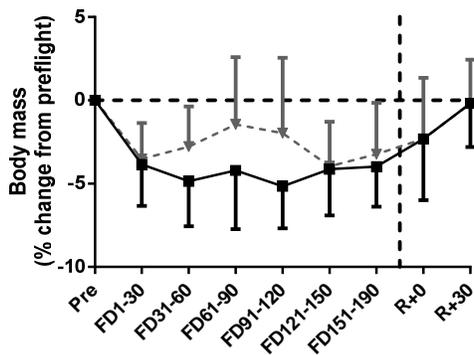


Figure 69. In-flight body mass measured by the BMMD (black lines) or SLAMMD (gray dashed lines), expressed as percentage change from before flight (N=25). Adapted from (60).

It is worth noting that loss of body mass during flight has lessened over time. Recent data from crewmembers who maintained body mass (assessed post flight) and performed heavy resistance exercise show the potential importance of being able to maintain body mass, in that these crewmembers came back leaner, with less fat, and had maintained bone mineral density at preflight levels (41, 58). This result is much better than the losses by crewmembers on Mir (29, 63), Shuttle (22, 71), or even early ISS missions (31, 578), when many individuals lost more than

5% of preflight body mass, an amount worthy of concern, and in many cases lost more than 10% of preflight mass. In crewmembers who must meet strict medical requirements for flight certification, including weight limits, a loss of 10% of body mass is clinically significant.

When SLAMMD and BMMD data for the same subjects were collected relatively close to each other, the results matched rather well, given the fact that for most of the data the 2 measurements were taken about 7 days apart. The correlation (**Figure 70**) had a slope of almost 1, indicating that BMMD and SLAMMD taken together produce very similar results (60). This analysis does not speak to the accuracy of either device, though, only saying that their measurements are consistently offset from each other by about 1.2 kg independent of body mass. Both BMMD and SLAMMD are less precise than the standard scale, but given the much more complex design of BMMD and SLAMMD, this decrease in precision might be expected and unavoidable. The decrease in precision of the BMMD and SLAMMD, compared to their precision reported in the literature (579, 580), could be due to human factors such as body position on these devices, reaction and movement during tests, or varying centers of mass, all of which can decrease the precision of the measurement.

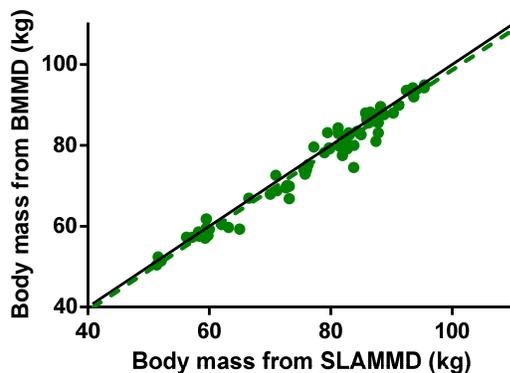


Figure 70. Comparison of in-flight body mass data from the SLAMMD and BMMD. For the linear relationship (dashed line), the Pearson  $r=0.9829$ . The solid black line is a line of unity for comparison. Adapted from (60).

The next steps to take to evaluate the SLAMMD and BMMD would be to properly assess accuracy by measuring a known reference mass on both devices during spaceflight. Until this test is completed, it is difficult to determine which instrument is accurately measuring the crewmembers' true in-flight mass. Further studies would also need to be done to clarify the time course of the change in body mass within the first 30 days of flight.

## 7. Vitamin D

Before the Nutrition SMO was implemented, it was well documented that vitamin D status (25-hydroxyvitamin D) decreased after long-duration spaceflight (30, 31, 39, 63). The absence of ultraviolet light during spaceflight diminishes vitamin D stores in the body, as observed during the 84-day Skylab mission (39) and more recent Mir missions (30, 63) and International Space Station (ISS) Expeditions (31). Reported supplement use was not related to 25-hydroxyvitamin D status. Despite the reported use of vitamin D supplements by some of the astronauts (average supplement use was  $3.0 \pm 2.8$  per week), the mean serum concentration of 25-hydroxyvitamin D for the ISS crewmembers in this study was about 25% less after landing than before launch.

At about the time that the Nutrition SMO 016E was implemented, vitamin D supplement recommendations to crews increased from 400 IU/d to 800 IU/d. The in-flight 25-hydroxyvitamin D data provide evidence that 800 IU/d is enough to maintain vitamin D status during long-duration spaceflight (**Figure 71**).

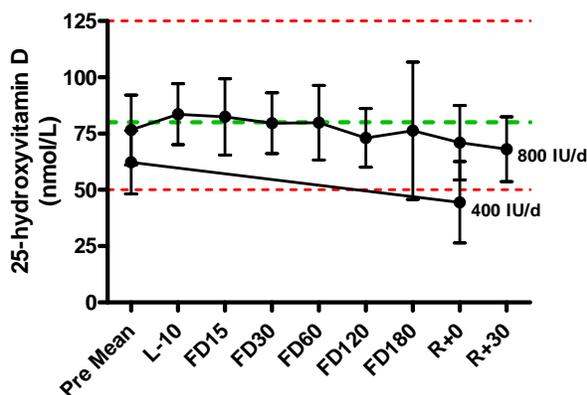
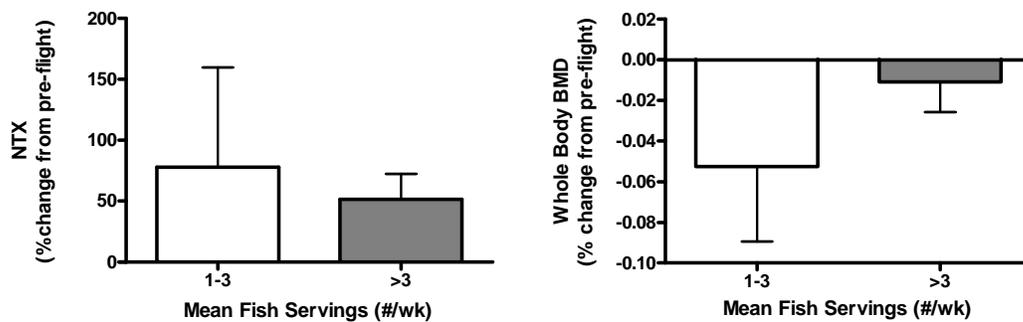


Figure 71. Pre- and postflight data from MedB8.1 show that vitamin D status decreased after long-duration spaceflight, despite vitamin D supplementation with 400 IU/d. In-flight data from SMO 016E showed that 800 IU/d was enough vitamin D to maintain status during long-duration spaceflight. Red lines depict Institute of Medicine-defined lower acceptable limits (with respect to bone health), and upper advised limit (279). 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 from (31, 41).

## 8. Fish and Bone

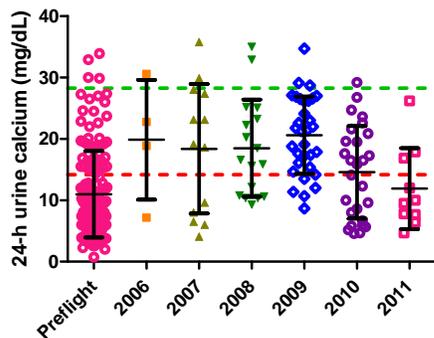
Data from ISS have documented a relationship between fish intake and bone loss in astronauts (that is, those who ate more fish lost less bone; Figure 72) (147). Related findings have also been shown in two sets of ground-based spaceflight simulation studies evaluating the role of omega-3 fatty acids, the fatty acid found in fish (147). In a bed rest study, the rate of bone breakdown was related to omega-3 fatty acid intake (again, more omega-3, less bone breakdown). In a series of cell culture studies, cells that break down bone were less active when omega-3 fatty acids were added (147). Although more detailed studies are required during spaceflight and using controlled dietary sources of omega-3 fatty acids from the space food system or supplements, these results have broad implications for all Americans, especially in light of the incidence of osteoporosis and other bone diseases, which affect millions of people.



**Figure 72.** Data from long-duration spaceflight showing that consumption of more servings of fish per week was related to less bone resorption after flight, as indicated by resorption marker urinary n-telopeptide (NTX) on the left, and greater bone density, as indicated by whole-body bone mineral density (BMD) on the right). Data are adapted from Zwart et al, 2010 (147).

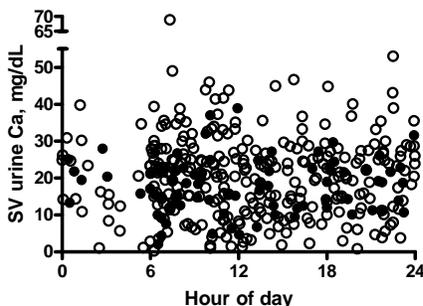
## 9. Urine Processor Assembly

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 Nutrition SMO urine volume and calcium excretion data from 24-h pools and single voids were closely examined (191). Urine 24-h 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. New recommendations for percentage of water to be recovered were made as a result of those findings. In 2012, when the data were reevaluated with Nutrition SMO data from an additional 10 subjects, it was clear that crewmembers in recent years are drinking more fluid than crewmembers in the past, and as a result urinary calcium concentration is lower (Figure 73).



**Figure 73.** Urinary calcium concentration before and during flight, by year of collection. Each symbol represents a 24-h urine collection (N=23, up to five 24-h collections/subject), mean  $\pm$  SD. The horizontal green dashed line represents a calcium concentration of 28.3 mg/dl, the expected calcium precipitation point for UPA water recovery at 70% (new set point), and the red dashed line represents calcium concentrations of 14.2 mg/dl, the expected calcium precipitation point for UPA water recovery at 85% (the original set point).

Suggestions for ways to mitigate high calcium concentrations included exclusion of the first morning void because that void is typically more concentrated than other voids throughout the day. Another suggestion was administration of low doses of bisphosphonates to crewmembers to reduce calcium excretion. A plot of in-flight single-void calcium data from the Nutrition SMO showed that neither of the suggested countermeasures looked promising (Figure 74).

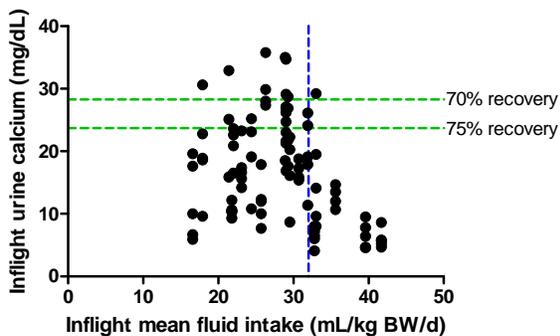


**Figure 74.** Concentration of calcium in urine voids relative to the time of day when they were collected. Filled circles represent crewmembers who were taking bisphosphonates during their mission. These data were evaluated to see if operational decisions (such as excluding the first morning void or prescribing bisphosphonates) could decrease the risk of calcium precipitating in the UPA. SV = single void (as opposed to 24-h pool data).

### *Risk Factor of Inadequate Nutrition*

From a plot of in-flight urine calcium concentration (from 24-h pools) versus in-flight mean fluid intake estimated from the food frequency questionnaire, with one exception, all voids were below 23.7 mg/dl, the cutoff point for a 75% water recovery from the Recycle Filter Tank Assembly when fluid consumption was greater than 32 mL fluid/kg body weight (**Figure 75**). For the 23 crewmembers in this analysis, that would average to 2.5 L/d fluid consumption (from food and beverages).

In September 2012, on the basis of these data, the ISS Program Control Board decided to increase water recovery from 70% to 74-75%. This will save an estimated 80 L of water per year, and 6-7 h of crew time (because of fewer tank changes). This recovery rate will also delay reaching the minimum reserve quantity of water (i.e., the point where water will need to be launched) by several months. The cost savings as a result of Nutrition SMO data will be tremendous.

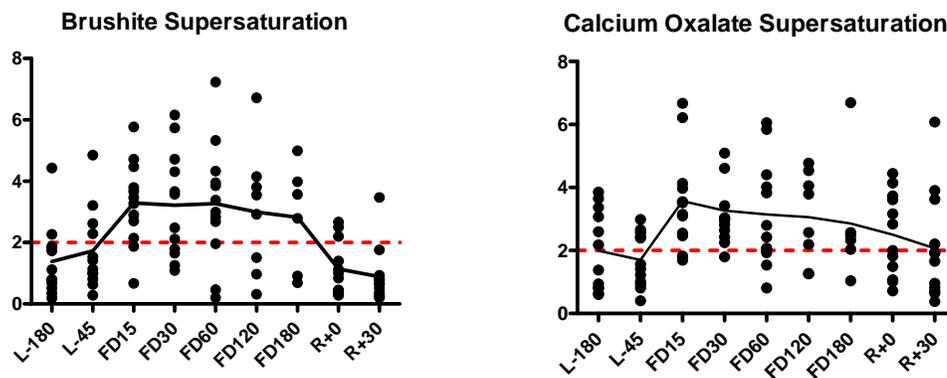


**Figure 75.** In-flight urinary calcium concentration versus in-flight mean fluid intake (estimated from the food frequency questionnaire). The green dashed lines represent the calcium concentration required to maintain 70% or 75% water recovery from the UPA system. 70% represents the conservative lower recovery rate adopted after system failure in 2009, and 75% the adjustment after revisiting these data in 2012. Figure from (191).

## 10. Renal Stone Incidence

The renal stone risk profile is determined from measured urinary oxalate, uric acid, citrate, and sulfate, and calculated supersaturation of calcium oxalate, brushite, sodium urate, uric acid, and struvite. Generally, the risk of renal stone formation is elevated during spaceflight (181). As with any spaceflight effect, some crewmembers are more affected than others. The graph below (**Figure 76**) is an important illustration of this. Some crewmembers had very high elevations in brushite or calcium oxalate supersaturation during spaceflight, while others had very low levels of supersaturation. Lifestyle, environmental, and dietary factors all greatly affect renal stone risk. Crewmembers who have an increased risk before spaceflight 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 spaceflight.

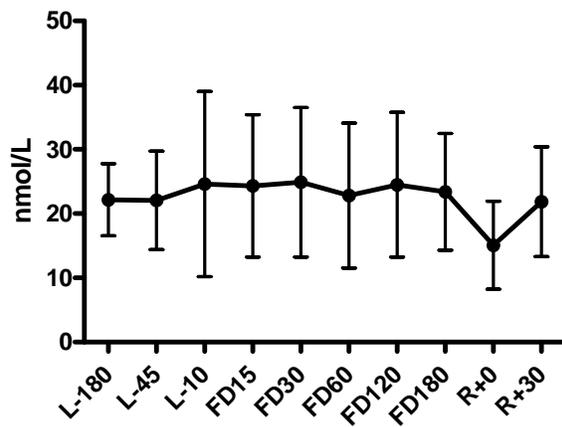
Medical Operations has asked to review pre- and in-flight renal stone data profiles from crewmembers who have experienced renal stones after flight. By looking at in-flight data profiles for these crewmembers, we can better identify the most important measures that can predict future renal stone incidence, and better determine which lifestyle or dietary factors are most important for lowering risk.



**Figure 76.** Estimated risk of brushite and calcium oxalate supersaturation from the Renal Stone Risk Profile. Each symbol represents an individual 24-h urine collection, and the solid black line represents the group mean. The red dashed line at 2 is the point above which the risk of supersaturation is greater than in the general population.

## 11. Testosterone

Testosterone, given its role in bone and muscle maintenance, is often considered either a potential mechanism for the musculoskeletal losses associated with spaceflight, or a potential countermeasure (581, 582). These considerations are based on very limited flight data from humans and animal models that suggest that testosterone levels are reduced during spaceflight, and on results from ground-based studies simulating spaceflight. Data from the Nutrition SMO 016E show that testosterone and related hormones are unchanged by real (Figure 77) or simulated weightlessness, apart from transient effects after flight. Nevertheless, 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 spaceflight on the human body. These data have been published (583).



**Figure 77. Total testosterone concentrations before, during, and after flight. Although circulating concentrations decreased significantly after flight (at R+0), no other time point differed significantly from the preflight mean. N=15. Data adapted from Smith et al (583).**

## 12. Vitamin K

Phylloquinone, undercarboxylated osteocalcin, and urinary  $\gamma$ -carboxyglutamic acid (GLA) are all measures of vitamin K status. Vitamin K is an enzyme cofactor for the production of GLA residues in specific proteins (GLA-proteins). GLA-proteins are involved in a number of regulatory functions, including bone mineralization. Osteocalcin is a GLA-protein that is synthesized in osteoblasts and is thought to have a role in the regulation of bone mineralization. Undercarboxylated osteocalcin provides a good measure of vitamin K functional status, especially with respect to bone.

Monitoring vitamin K status during flight for the Nutrition SMO has documented a lack of evidence that vitamin K status is decreased during spaceflight, a finding earlier reported from a single case study on Mir (339, 340). Nutrition SMO data from the first 15 participating crewmembers showed no major changes in phylloquinone, urinary GLA, or undercarboxylated osteocalcin (**Figure 78**) (341). This was an important finding, to document that a vitamin K countermeasure to mitigate a vitamin K deficiency during spaceflight is not needed and therefore likely would not have an effect on bone.

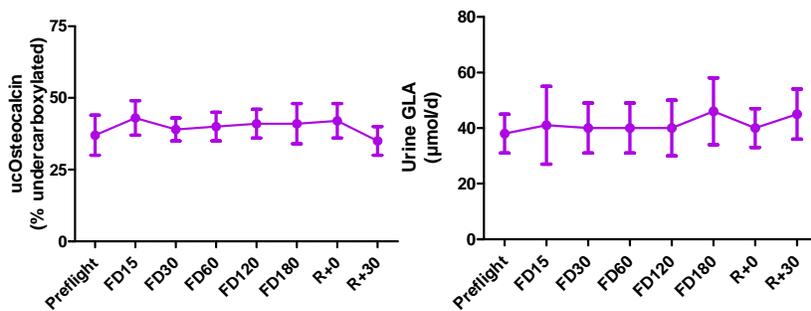


Figure 78. Effect of long-duration spaceflight on vitamin K status of 15 ISS crewmembers. Undercarboxylated osteocalcin (left) and urinary  $\gamma$ -carboxyglutamic acid (GLA, right) are shown here. No significant changes resulted from spaceflight (341).

## 13. Summary

The findings to date from the Nutrition SMO have shed light on a number of metabolic issues that are important for human long-duration spaceflight, and as more data are collected we will continue to review, present, and publish the findings. Given the comprehensive nature of the Nutritional Status Assessment protocol, there are likely many lessons to be learned, beyond those already identified. These data will be a valuable resource for years to come.

## **VIII. N4: Does mission architecture and/or available countermeasures impact nutritional status of crewmembers during spaceflight?**

Many aspects of space missions impinge on nutrition. These include the more obvious aspects, such as mission duration and the vehicle itself, but they also include other factors. Spacewalks, or extravehicular activities (EVAs) can have an impact on nutrition, from exposure to high oxygen environments to the fact that one cannot consume food during an EVA. The type of food system itself can also affect nutrition. Countermeasures for one physiological system can often have a negative effect on another. For one clear example, the exercise equipment and protocols will alter energy and nutrient requirements. Drug-nutrient interactions are a major concern, along with the common suggestion that nutritional supplements be provided to “eliminate” any concerns about nutrition. Many of these factors are described in this section, and others can be described only after future exploration missions have been defined.

### **A. Extravehicular Activity**

#### **1. Overview**

Extravehicular activity (EVA) 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. As a result of the requirements of EVA preparation and EVA itself, crewmembers can go without food for up to 8-10 hours. Recommendations for EVA nutrition 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 (4). This recommendation 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 300-500 kcal, with about 70-100 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, other 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 concern. Crewmembers lose 6-8 ounces of fluid/h (177-237 mL) during an EVA. The current EVA suit contains a 24- or 32-ounce drink bag (710 or 946 mL). 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.

Although the issue of nutritional support during EVA was reviewed only briefly at the 2005 Standards and Operating Bands meeting, no recommendations were made to change the 2000 guidelines. As suits are developed for exploration mission transit and planetary EVAs, following the suggestions above would alleviate problems deemed too complicated to solve with the suit used on the Shuttle and ISS.

Along with food and fluid issues associated with EVA, the hyperoxic environment also has the potential to cause additional damage to the body. The prebreathe protocol for U.S. astronauts typically includes a 2.5-h prebreathe of >95% to 100% oxygen (584) to reduce the risk for decompression sickness. After the 2.5-h prebreathe period, astronauts are typically exposed to hypobaric 100% oxygen for 6-8 h during EVA. Studies from saturation dives show that oxidative damage occurs under similar conditions (358). Judging by the results of numerous ground-based studies with hyperoxia, including data from a NASA Extreme Environment Mission Operations 14-day saturation dive (358), the potential exists for nutritional countermeasures to mitigate some of the oxidative damage (585-587).

## **2. Gaps**

Because proposed plans for lunar EVAs will be similar in duration (8-10 h) but more frequent (2-3 times per week) than current ISS EVA schedules, there is a clear need for development of nutritional support during EVAs. A nutrition support system will need to fit the lunar suit design. 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 due to a high partial pressure of oxygen in the suit.

## **B. Supplements**

### **1. Overview**

Along with the issue of nutrient requirements for space travelers and the use of nutrients as countermeasures to the negative effects of spaceflight, the issue of supplements arises. Supplements may allow individual nutrients to be obtained when the diet cannot meet the body's needs. However, foods but not supplements provide non-nutritive substances and psychological benefits, and supplements may have side effects, interact with medications, and be treated differently by the body when they are removed from the matrix of nutrients in a whole food. The only supplement NASA recommends for astronaut use during spaceflight is vitamin D.

### **2. Supplements versus Whole Foods**

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 spaceflight, especially oxidative damage and radiation-induced cancer risk. The benefits of supplements are such that individual nutrients can be obtained when the diet cannot meet the body's needs. Drawbacks to supplements include their lack of beneficial non-nutritive substances, potential for side effects, interactions with medications, and different treatment by the body when they are removed from the matrix of nutrients in a whole food.

It is generally agreed that nutrients should be provided to astronauts in standard foods instead of supplements (4, 5, 43, 97, 588). Natural foods provide valuable non-nutritive substances, such as fiber, carotenoids, and flavonoids, as well as a sense of palatability and psychological well-being that will be important during long missions. The need for more detailed information about the "psychophysiology of hunger and eating" was noted decades ago during the early space programs (13), 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.

Isolated nutrients may not provide the same protective effect on bone as they would in the matrix of the whole food. Omega-3 fatty acids have different effects on vasodilation, depending on whether they are supplied as a supplement or in a whole food (589, 590). 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 much more than pure lycopene alone (591, 592).

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 ( $\beta$ -carotene in the CARET study) or increased risk of stroke (268, 593-595).

When many nutrients are provided as oral supplements, they are not metabolized by the body as they are when in foods (596). Changes in bioavailability and metabolism of nutrients can increase the risk of malnutrition.

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.

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.

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 U.S. crewmembers on ISS. Early crews received 400 IU vitamin D<sub>3</sub> per day (31), but recently this was raised to 800 IU per day of supplementation (97); this level allows maintenance of serum 25-hydroxyvitamin D around 75 nmol/L (41).

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 (3-5 y), and their potential interaction with pharmaceuticals. Most importantly, supplements will need rigorous testing to demonstrate that the level used is not toxic to body systems, and will need close monitoring during flight to ensure that their interactions with the spaceflight 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 (597). 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 (598, 599).

### **3. Gaps**

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, especially considering the long proposed shelf-lives of food items for exploration-class missions. Supplements, if they are recommended, would need to be tested in ground models to test their efficacy in maintaining nutrient status, their stability over a long duration (3-5 y), and their potential interaction with pharmaceuticals, as well as to identify their side effects on all body systems.

## **C. Nutrient-Drug Interactions**

### **1. Overview**

While a specific gap has not been identified regarding nutrient-drug interactions, this is clearly an area requiring additional study. An understanding of interactions between pharmacotherapeutic agents and nutrients is necessary to implement safe and effective medical care and clinical intervention operations for astronauts on long-duration missions. The most common studies of interactions between pharmacological agents and nutrients concern their effects on a nutrient 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 (600). Cytochrome P450 enzymes are unique in their ability to use a wide range of substrates (601). 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 spaceflight and the space food system: dietary factors, nutrient metabolism, monoamine oxidase inhibitors, and antacids and proton pump inhibitors.

### **2. 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 (602). Phase II requires glucose, sulfur-containing amino acids, and glutathione (602).

The effects of nutrients on drug metabolism have been well studied in animal models; however, relatively few dietary factors have been studied in humans (602, 603). 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 (604, 605). 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 (606, 607). 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 (602, 603, 608-611). 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 (603). 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 toxic effects of the drug (612). 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.

### **3. 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<sub>3</sub>. Previtamin D<sub>3</sub> undergoes an isomerization to form vitamin D<sub>3</sub>, a biologically inactive compound. CYP27A is a mitochondrial mixed-function oxidase that is responsible for hydroxylating vitamin D<sub>3</sub> to form 25-hydroxyvitamin D<sub>3</sub> (613). CYP3A4 has been found to be a 25-hydroxylase as well (614). CYP27B converts 25-hydroxyvitamin D<sub>3</sub> to 1,25-dihydroxyvitamin D<sub>3</sub>. 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<sub>3</sub> 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 (615). Certain drugs are known to activate CYP24 activity, including rifampin, isoniazid, and phenobarbital (616, 617). Several studies show a relationship between the use of these drugs and osteomalacia (618, 619), 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 (620, 621). Inducers of CYP1A2 (cigarette smoke, cruciferous vegetables, broiled beef, rifampin) may affect vitamin A metabolism.

#### **4. 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 (602). Fermented foods and protein-rich foods that have begun to spoil are rich in phenylethylamines (602).

#### **5. 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<sub>12</sub>, vitamin C, iron, and vitamin A (602, 622, 623). Antacids can precipitate folic acid at a pH greater than 4.0, thus rendering it insoluble and not available for absorption (624). A high pH also affects thiamin bioavailability because the vitamin is not stable at high pH (602). 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 (623). These changes are observed mostly in subjects who are infected by *Helicobacter pylori* and are taking proton-pump inhibitors (623).

Vitamin B<sub>12</sub> 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<sub>12</sub> 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 (622). This would be particularly harmful if vitamin B<sub>12</sub> stores were low before therapy began.

## **6. Summary of Pharmacology and Drug-Nutrient Interactions**

Currently no data are available that pertain to specific drug-nutrient interactions during spaceflight. 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.

## **7. Gaps**

Currently, no data are available regarding specific drug-nutrient interactions during spaceflight. The main concerns for a long-duration mission involve the 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.

**IX. N6: What impact does the spaceflight environment have on oxidative damage? N15: We need to identify the most important nutritional factors for oxidative damage during spaceflight.**

**A. Overview**

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 (498, 501, 625-627). After spaceflight, 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 (326).

**B. 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 (628, 629). Future vehicles or exploration habitats will likely operate at 8.0 to 8.2 psia and 32% to 34% oxygen with the balance nitrogen (629). 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.

**C. Extravehicular Activity**

Extravehicular activity, 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 (4). 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 nutritional 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.

#### **D. 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 findings from the 1950s suggesting that short-lived reactive intermediate molecules like ROS are present in skeletal muscle after exercise (630). Since then, numerous studies have supported a role of ROS in skeletal muscle fatigue (630-632). Mitochondria are the major source of ROS in muscle cells, where a fine balance of ROS exists between maximizing force and minimizing fatigue (625). Antioxidant-mediated depletion of ROS from unfatigued muscle yields decreased production of skeletal muscle force (633). On the other hand, excess ROS can be detrimental in terms of fatigue. ROS can denature proteins directly associated with the sarcoplasmic reticulum  $\text{Ca}^{2+}$  release mechanism (634), 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 (635). Furthermore, decreased antioxidant status lowers exercise capacity and increases onset of fatigue in human and animal studies (630, 632).

Astronauts perform extensive upper-body exercise during EVA, 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.

#### **E. Radiation Exposure**

Astronauts are exposed to highly ionizing radiation, in addition to secondary radiations resulting from interactions 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 reactive oxygen species (ROS), and oxidative damage induced by a bystander effect (123, 636, 637). 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 (638).

#### **F. Oxidative Damage Markers During Spaceflight and in Ground Analogs**

Evidence for oxidative stress resulting from spaceflight exposure exists in multiple tissues, including ophthalmic tissue (273, 639); in urinary and blood biomarkers of damage to DNA, lipids, and protein (31, 327, 498, 640); and in gene expression (641, 642). Plasma malondialdehyde (MDA), 8-iso-prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\alpha$ ), and urinary 8-hydroxy-2'-deoxyguanosine (8OHdG) have been measured during and after flight as indicators of lipid peroxidation (MDA and PGF<sub>2</sub> $\alpha$ ) and DNA damage (8OHdG) (29, 327). A significant elevation of urinary 8OHdG has been noted after long-duration missions (Mir and ISS) (31). These data are supported by results from the ground-based analog NEEMO, in which crewmembers underwent 10- to 14-day saturation dives (358, 507). Urinary PGF<sub>2</sub> $\alpha$  was significantly decreased during flight but was elevated about 2.5-fold after flight (327), and plasma MDA was increased both during and after flight (327). 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 (643).

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 (644, 645), but evidence exists that downregulation of antioxidant defense systems occurs during spaceflight (646). Along with increases in markers of oxidative damage and decreases in antioxidant defense systems, a decrease in total antioxidant capacity also occurs.

#### **G. Fruits and Vegetables**

A recent effort has been made to allow for healthier foods to be flown for ISS crewmembers. One major goal of this effort is to provide more fruits and vegetables, which have documented effects on overall health, on specific systems, and on oxidative damage itself. While teasing out specific effects, most believe the antioxidant properties of fruits and vegetables are integral to their success in disease mitigation, although individual nutrient supplement trials generally fail to help enlighten this phenomenon. Diets high in fruits and vegetables have been documented to reduce oxidative damage (647). Dietary effects on microbiome, specifically fruits and vegetables, have also been identified (648-650). This has not been studied during flight, and hopefully this new effort will help fill this gap, and inform our understanding of both food and nutrition related issues.

#### **H. Antioxidants and Related Nutrients: Selenium, Vitamin E, Vitamin C**

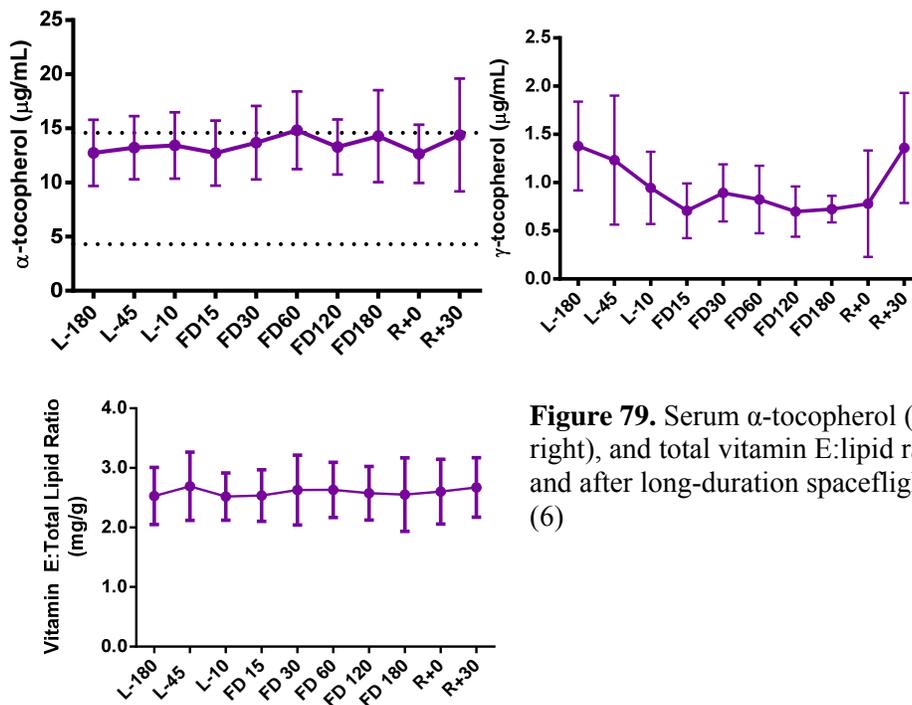
Selenium has been shown to play a role in the maintenance or induction of cytochrome P450, pancreatic function, DNA repair, enzyme activation, immune system function, and

detoxification of heavy metals (651). 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 (31). 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 (651). 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 ( $\alpha$ -,  $\gamma$ -,  $\delta$ -, and  $\beta$ -tocopherol) and 4 tocotrienol derivatives ( $\alpha$ -,  $\gamma$ -,  $\delta$ -, and  $\beta$ -tocotrienol) (321). The tocopherols that are most abundant in biological systems are  $\alpha$ - and  $\gamma$ -tocopherol, but small amounts of  $\delta$ -tocopherol and  $\gamma$ -tocopheryl quinone are also present. About 90% of the tocopherol found in human plasma is in the form of  $\alpha$ -tocopherol (322). After ISS crewmembers had spent 4 to 6 months in space, their plasma  $\gamma$ -tocopherol was 50% less than preflight levels (31), but no change in  $\alpha$ -tocopherol, or vitamin E to lipid ratio, occurred in these subjects (Figure 79).



**Figure 79.** Serum  $\alpha$ -tocopherol (top left) and  $\gamma$ -tocopherol (top right), and total vitamin E:lipid ratio (bottom) before, during, and after long-duration spaceflight. All data are mean  $\pm$  SD. (6)

Oxidative stress can increase in microgravity and high-radiation environments (326-328), 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 (329), and it may be necessary to provide enough vitamin E for astronauts' blood levels of the vitamin to be higher during spaceflight 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 (598, 599, 652, 653).

The term "vitamin C" actually refers to 2 different compounds, ascorbic acid and dehydroascorbic acid, both of which have activity against scurvy (654). 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 (654). Vitamin C also provides antioxidant protection by returning  $\alpha$ -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 (654).

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 (322).

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 (655), the significance of this association was marginal when data were adjusted for potassium intake. This suggests that vitamin C may be a secondary factor related to fruit and vegetable reduction in bone loss (656-659), 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 (660-663). Vitamin C has been related to cataract and cancer incidence (664-666), 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 (3). Toxicity of vitamin C leads to gastrointestinal distress, and has been reported in subjects consuming more than 1000 mg/d (664).

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 (356, 667).

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 (370). Vitamin C is also unstable when exposed to light or heat (370), and in irradiated foods (371, 372). Salem (372) 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 (373). 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 (Cooper et al, unpublished data). 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.

A major concern for spaceflight 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 (374, 375). Just as important to consider, however, is the possibility that vitamin C could induce DNA damage. Cai and colleagues (375) 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 (376). 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 spaceflight-induced stress on vitamin C, an evaluation of intake requirements needs to be made.

**X. N7.1: We need to identify the most important nutritional factors for musculoskeletal health.**

**A. Bone and Calcium**

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 spaceflight. Almost as soon as weightlessness begins, 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 U.S. space agencies). This number of crewmembers has allowed the effects of spaceflight on bone physiology to be documented, along with the testing of countermeasures aimed at counteracting bone loss.

Although this topic has been reviewed extensively (314, 418, 419, 421, 424, 457, 463, 668), we attempt to provide here an overview of bone and calcium changes that occur during spaceflight, 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.

**1. Bone Loss**

Bone is lost during spaceflight, primarily from weight-bearing bones. This was first documented on Skylab missions (413, 669), and later on Mir (417, 426, 433, 670) and ISS missions (41, 58, 561). 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 (417, 418, 425, 426), 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 (419), 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 (116, 117, 181, 182, 671). The subject-to-subject variability seems a characteristic of spaceflight-induced bone loss (424), and as more data are accrued, may provide insight to find a means to mitigate this loss (672).

Long-term follow-up data on bone recovery are far from complete (420, 458). However, if the rate of postflight recovery estimated from biochemical data is also assumed to be constant

(reasonable according to ground-based (436) and flight (30, 63) data), then the rate of recovery is about +100 mg/d (30, 63). 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 to 9 months after flight (420, 672). For longer exploration missions, however, the usefulness of these assumptions comes into question, as spaceflight 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.

## **2. 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, or both, of these processes can be problematic.

Bone resorption increases during spaceflight. 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; they 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 crosslink molecules. Increased collagen crosslink excretion, and thus bone resorption, has been shown to be clearly increased during spaceflight (30, 63, 431-433, 673, 674). Earlier studies showed that plasma concentrations of a similar but more easily confounded marker, hydroxyproline, were elevated during short-duration Shuttle flights (673) and Skylab missions of longer duration (39, 94, 434). Calcium tracer kinetic studies also provided data indicating that bone resorption increases about 50% during flight relative to preflight (30, 63) levels.

Bone formation either remains unchanged or decreases during spaceflight (30, 63, 425). As indicated by serum concentrations of bone-specific alkaline phosphatase (BSAP) and osteocalcin, bone formation was unchanged during Mir flights, but increased 2 to 3 months after landing (30, 63). Trends toward decreased levels of bone formation markers were noted in 2 Mir studies with 1 subject each (432, 433). The results of studies, using calcium tracer techniques, of bone formation in 3 Mir crewmembers (30, 63) were equivocal (formation was unchanged or decreased). Together, increased resorption and decreased or unchanged formation yield an overall negative calcium balance (30, 63).

The exact triggering mechanism for these changes in bone metabolism during spaceflight 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 (30, 63, 674). 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 (316, 435).

Spaceflight analog studies (such as bed rest) with humans have shown qualitative effects on bone and calcium homeostasis similar to those shown in flight studies (424, 675), with quantitative effects generally being of smaller magnitude. Effects include loss of bone mass (436-438, 676), decreased calcium absorption (439), increased urinary excretion of calcium and biochemical markers of resorption (81, 357, 415, 439-446, 667, 676, 677), increased risk of renal stone formation (115, 443, 444), and decreased serum concentrations of PTH (81, 296, 357, 441, 667, 678) and 1,25 dihydroxyvitamin D (296, 439, 441, 447, 667).

That bone resorption increases during bed rest has been shown by histomorphometry (438, 448) and measurement of biochemical markers. Excretion of hydroxyproline (439, 445, 677) increases during bed rest, and excretion of collagen crosslinks (81, 296, 357, 431, 439, 667, 676, 678) is elevated about 50% above control levels, compared with the increase of greater than 100% during spaceflight (30, 63, 431).

The concentrations of biochemical markers indicate that bone formation is unchanged during bed rest (81, 296, 357, 437, 439, 667, 678), but histomorphometry data from bone biopsies show that bone formation decreases (438, 441, 448). 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 (437, 439). Recent evidence indicates that with bed rest studies of longer duration (eg, 90 d), bone formation markers tend to increase (357, 676). In these initial publications, 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 (421).

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 (679, 680) and the availability of commercial immunoassays in the 1990s (681, 682) allowed resolution of this matter: bone resorption increases during spaceflight and bone formation decreases or does not change significantly, substantiating that spaceflight disrupts the balance between bone resorption and formation, which can lead to a net loss in bone mass.

### **3. Bone Loss Countermeasures**

#### **a. 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 flight (462, 675, 683, 684). With respect to bone specifically, treadmill and cycle exercise devices available on Mir did not prevent bone and calcium loss (30, 63, 417, 685, 686). 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 (687).

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) (296, 564, 688-693). Most evidence, from the general scientific literature as well as spaceflight analogs, pointed to resistance exercise as the most likely means of counteracting bone loss

during spaceflight. 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 80).

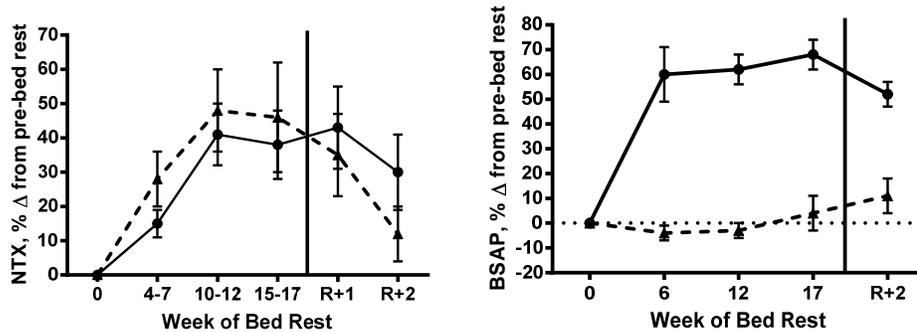


Figure 80. Bone resorption (as indicated by urinary n-telopeptide [NTX], left panel) and bone formation (as evaluated by serum bone-specific alkaline phosphatase [BSAP], right panel) during 17 weeks of bed rest with (solid line) or without (dashed line) heavy resistance exercise. Data are expressed as percentage of pre-bed rest values, and are mean  $\pm$  SD. The vertical line separates the bed rest and post bed rest periods. Data adapted from (564).

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 BSAP (81) (Figure 81).

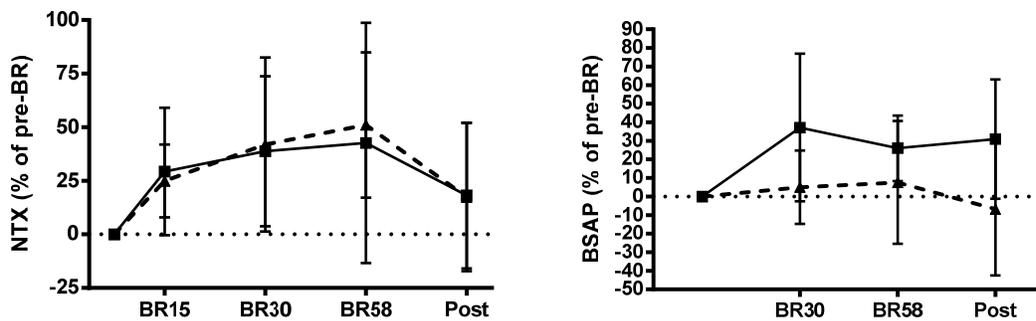


Figure 81. Bone resorption (as indicated by urinary n-telopeptide, left panel) and bone formation (as indicated by serum bone-specific alkaline phosphatase, right panel) during 60 days of bed rest with (solid line) or without (dashed line) a combination of resistance exercise and supine treadmill/LBNP exercise. Data are expressed as percentage of pre-bed rest values, and are mean  $\pm$  SD. Data adapted from (81).

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 (41, 561). 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.

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 (41, 562). Comparing crewmembers exercising with each device 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 82), and maintained bone mineral density in most regions and in whole-body scans (Figure 83), when assessed by DXA (41, 58).

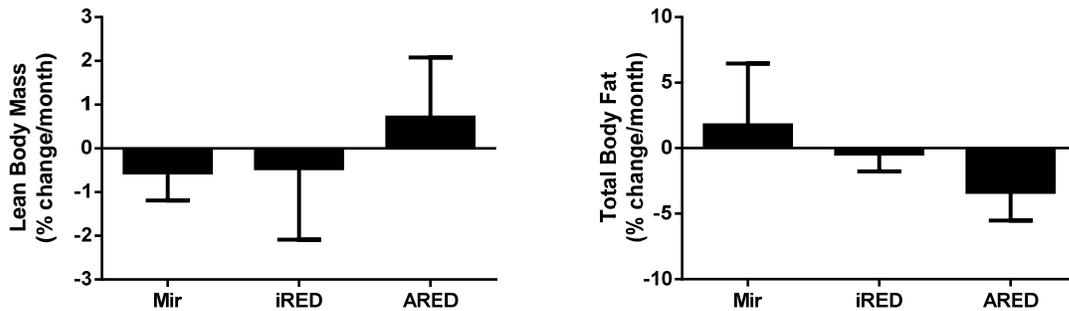


Figure 82. Body composition changes (left panel, lean body mass; right panel, total body fat) in astronauts on Mir and ISS missions. ISS crews had access to either iRED or ARED exercise devices. Data are expressed as percentage change per month of flight. Figure adapted from (41).

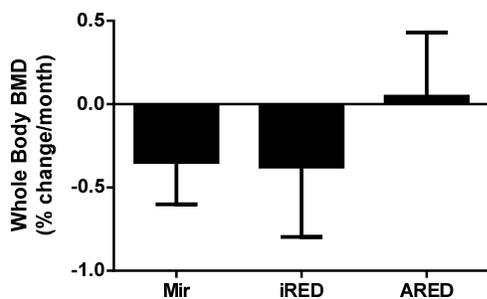


Figure 83. Bone mineral density loss in astronauts on Mir and ISS missions. ISS crews had access to either iRED or ARED exercise devices. Data are expressed as percentage change per month of flight. Figure adapted from (41).

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 (58) (Figure 84), 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 (41). 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 spaceflight that diet and exercise can maintain bone mineral density on long-duration missions.

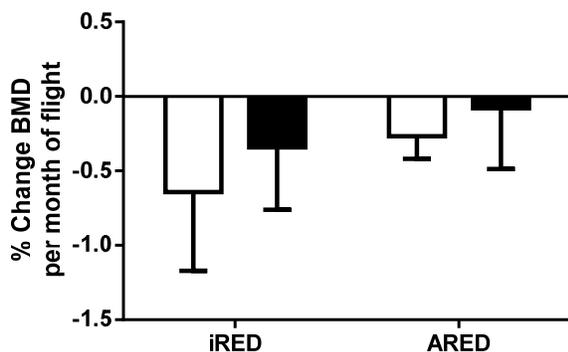


Figure 84. Bone mineral density (BMD) loss after flight in men (N=33, open bars) and women (N=9, solid bars) who used either the iRED or ARED exercise device. Data are expressed as percentage change per month of flight. Figure adapted from (58).

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 (41). In the flight study, as published, a significant ( $P < 0.001$ ) increase occurred (41), and in bed rest, heavy resistance exercise 6 days a week led to dramatic increases in bone formation markers (564). In another study with resistance exercise every other day (combined with a treadmill protocol), subjects had roughly half the bone formation response (81) of subjects in the first study (564). 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 (58). 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 (41) 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) (564).

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 spaceflight. 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.

**b. 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 (694). One hour per day of 1 G<sub>z</sub> exposure at the level of the heart and 2.5 G<sub>z</sub> at the feet was beneficial for some systems (eg, cardiovascular, muscle) (694-696), but this regimen did not have any effect on bone or calcium metabolism (678, 697). Although greater durations, increased *g* forces, or combining centrifugations with exercise protocols have been proposed, these have not yet been extensively tested (694, 698).

The assumption that it is lack of gravity that stimulates bone loss during spaceflight provided the impetus for proposing replacement of gravity by centrifugation (“artificial gravity”) as a countermeasure for multiple body systems (699, 700), particularly for bone. The statement has been made that it is known that unit (Earth) 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 (701). Some of the artificial-gravity studies have relied on short-radius centrifuges (702), others on rotating exercise devices (698, 703) 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 (704-706). As noted above, 1 hour per day of centrifugation resulting in 1 G<sub>z</sub> at the heart and 2.5 G<sub>z</sub> at the feet was ineffective for bone (678). The optimal artificial gravity prescription for bone, including dose, duration, and frequency of centrifugation, remains to be clarified (707), along with its potential impact on nutrition and related systems (708).

**c. Vibration**

Protocols for exposure to vibration, of both high and low frequency, have also been proposed and tested in spaceflight analogs. Although low-frequency protocols showed promise for protecting bone in both animal and ambulatory human studies (709-711), the beneficial findings were more limited when testing occurred during head-down-tilt bed rest (712). Higher frequency vibration, often referred to as resistance vibration exercise, has generally shown positive effects on bone and muscle during bed rest (713-716). Debate continues over the potential of this countermeasure protocol, amid safety concerns about neuromuscular issues with repeated vibration exposures.

**d. 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 (438, 462, 689, 690, 692, 717-724). 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 spaceflight. As noted above with exercise, given that the bone loss of bed rest is about half that of spaceflight, 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 spaceflight-induced increase in bone resorption cannot fully, or chronically, be mitigated by bisphosphonates (725, 726).

Endocrine therapies, including exogenous calcitonin administration (462, 677), have also been attempted, albeit unsuccessfully. In animal models, testosterone has also been suggested as a bone loss countermeasure (581, 727) on the basis of limited data showing a reduction in testosterone concentrations during flight in human, animal, and cellular models (728-734). Reduction of testosterone, however, has recently been shown to likely not be a concern during spaceflight. See section VI.B.11, “Testosterone,” for a more detailed discussion of these data.

#### e. Nutritional Countermeasures

One of the most obvious nutritional countermeasures—providing calcium—does not protect against bone loss (449). This result is likely related to the decreased calcium absorption seen during bed rest (439, 697) and spaceflight (30, 63, 674), 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 (735). Combination therapy with calcium and phosphorus was also unsuccessful at mitigating bone loss and hypercalciuria (677).

Omega-3 fatty acids have been shown to protect bone, in the general population (144-146) and animal studies (736) as well as in spaceflight analog studies, including bed rest (Figure 85) and cell culture (147). While omega-3 fatty acids have not been studied in a controlled fashion during actual spaceflight, a positive correlation was found between fish intake and bone loss in astronauts (Figure 85) (147). 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 spaceflight. Studies showing positive effects of omega-3 fatty acids typically look at intake of fish or other food sources of these nutrients (148-150). Studies of fish oil supplements added to typical diets often fail to document any benefit (151-153), thus highlighting the need for dietary modification, and not simply supplementation.

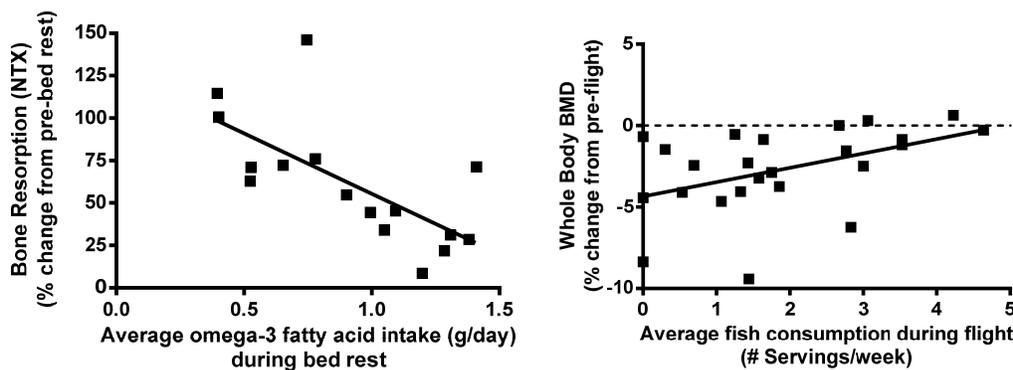


Figure 85. Relationship between omega-3 fatty acid consumption and bone resorption during bed rest (left panel), and relationship between fish consumption and percentage change in whole-body bone mineral density at landing in astronauts (right panel). Figures adapted from (147).

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 (98). These are discussed in detail in other sections of this book.

#### **f. Fruits and Vegetables**

Apart from nutrients, food patterns can obviously have effects on bone (and general) health. An ongoing effort to provide healthier foods for astronauts included the specific goal of increasing fruit and vegetable intake. Population-based and even smaller controlled studies have shown extensive benefits for the inclusion of 5 to 6 servings of fruits and vegetables per day (737). From a bone perspective, higher fruit and vegetable intake has been associated with increased bone mineral density (738-740) and lower risk of hip fracture in both men and women (741-744). Higher dietary quality (e.g., a diet rich in fruits and vegetables) has been shown to be associated with less bone breakdown and greater bone mineral density, and lower dietary quality (a diet rich in highly processed foods) with the opposite effects (745). Fruits and vegetables have even been posited as a potential alternative osteoporosis therapy (746), and at a minimum, as being beneficial for healthier bones during aging (747). Beyond bone, higher fruit and vegetable intakes have been associated with prevention of chronic diseases, including cardiovascular disease (748-750) and cancer (748, 749), as discussed in later sections.

Specific nutrients found in fruits and vegetables have also been studied, in attempts to identify key ingredients. Fruits and vegetables rich in carotenoids and other flavonoids have been associated with lower hip fracture risk (751). Lycopene, found in abundance in tomato-rich foods, has also been associated with protection of bone (752) and reduction of fracture risk (753). Lycopene also has been shown to reduce oxidative damage and bone breakdown (752, 754). This association of diet, oxidative damage, and bone on Earth is analogous to the association of iron, oxidative damage, and bone health in spaceflight (498) and ground-based analogs (514). Radiation, likely acting through oxidative damage, also has been identified as a risk factor for bone loss (567, 568).

## **4. Nutrients and Bone Health**

### **a. 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 spaceflight (435, 755). 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 (39, 94, 413, 415, 427, 428, 756) and Mir (30, 63) missions. During the 84-day Skylab 4 mission, calcium balance was  $-200$  mg/d (94, 429, 756). Increased urinary and fecal calcium excretion accounts for most of the calcium deficit (30, 39, 63, 94, 181, 413, 415, 428). 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 (30, 63, 94, 456). 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 (455, 757, 758), around the duration of many ISS missions.

### **b. 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  $\gamma$ -carboxyglutamic acid (GLA).  $\gamma$ -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 (333). 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 loss countermeasure (759).

Data from 11 U.S. astronauts on ISS Expeditions 1 to 8 (mission durations of 128 to 195 days during 2000-2004) revealed that on landing day their serum phylloquinone (vitamin K<sub>1</sub>) was 42% lower than it was before flight, whereas urinary GLA did not change (31). In another study, undercarboxylated osteocalcin was elevated (a sign of vitamin K insufficiency) as early as the 8th day of spaceflight, and remained high during 21- and 180-day missions (339). Studies on the EuroMir 95 mission showed that markers of vitamin K status were decreased after 12.5 weeks of spaceflight, and vitamin K supplementation (10 mg/d for 6 wk) reversed these effects (340). Vitamin K supplementation elevated GLA and decreased undercarboxylated osteocalcin, suggesting that vitamin K status was lower during spaceflight and was improved by supplementation (339, 340). 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 spaceflight. In-flight data from 15 crewmembers on Expeditions 14 to 22 showed no major changes in phylloquinone, urinary GLA, or undercarboxylated osteocalcin (341). Phylloquinone data from those 15 crewmembers (341) plus an additional 11 crewmembers on Expeditions 23 through 31 are shown below (Figure 86). 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 spaceflight is not needed and therefore likely would not have an effect on bone.

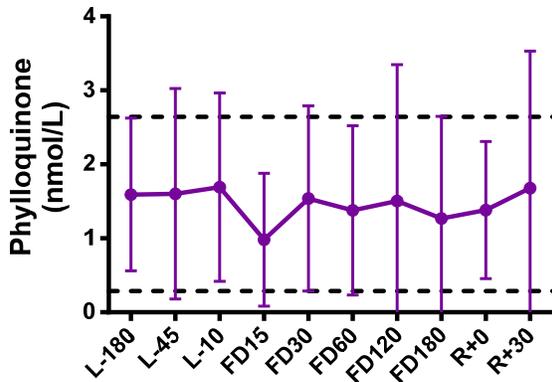


Figure 86. Serum phylloquinone before launch (launch minus 180 d, or L-180, L-45, and L-10), during flight (flight days 15, 30, 60, 120, and 180), on landing day (R+0), and 30 days post landing (R+30). The dashed lines indicate the normal range for phylloquinone. Data are mean  $\pm$ SD, N=26 (6). Data and N are expanded from the original publication of these findings (341).

### c. Phosphorus

Phosphorus is an important component of cell membranes and bone mineral. Phosphate accounts for about 60% of bone mineral (406), and most (85%) of the body's extracellular phosphorus is in bone (760). After ISS missions, urinary phosphorus excretion was about 45% lower than preflight excretion (31).

Excretion of phosphorus during untreated bed rest was not changed (357) from ambulatory conditions. An earlier study of 3 subjects revealed increased urinary phosphorus and negative phosphorus balance (445). 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 (677).

High phosphorus intake, relative to calcium in particular, can have effects on many systems, including bone, kidney, cardiovascular, and others (761-763). Ideally, the ratio of calcium:phosphorus should be around 1.0, or higher. The ISS requirements match this, with a notation that phosphorus:calcium ratio should not exceed 1.5 (2, 5, 97), 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 (425). To date, phosphorus intakes have been higher than desired. The standard menu has a P:Ca ratio of 1.8 (2); the ratios of actual intakes have been slightly lower than that, but still greater than 1.5 (Figure 87). Bed rest studies have tended to have P:Ca intake ratios closer to 1.0 (unpublished observations).

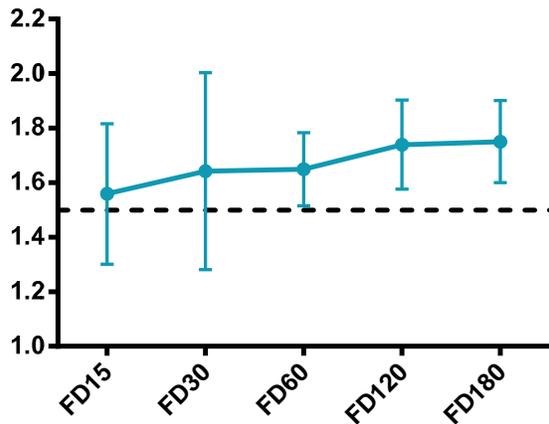


Figure 87. Dietary phosphorus:calcium ratio in ISS crewmembers during flight (approximate flight days 15, 30, 60, 120, and 180). The dashed line indicates a ratio of 1.5. Data are mean  $\pm$ SD, N=9.

#### d. Magnesium

Magnesium is the fourth most abundant cation in the body, and more than half of the body's magnesium is in bone (473). 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 (473).

Decreased urinary magnesium after flight, relative to before flight, seems to be a hallmark of spaceflight (39, 495). Serum magnesium trends downward during and after flight, as seen with Skylab and Shuttle crews (2, 31, 39, 208, 474). After crewmembers had spent 4 to 6 months in space on ISS, their urinary magnesium was about 45% less than it was before flight (31).

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 (475). 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 (690), with little effect of exercise or bisphosphonate. Recent studies conducted in the U.S. have shown a decrease in magnesium excretion in short- and long-duration bed rest (356, 357).

Magnesium shows promise for reducing the risk of renal stone formation (764). In ground-based studies, potassium-magnesium citrate has proven effective in reducing bed rest-induced risk (115). Potassium citrate (KCit) has been successfully tested during ISS missions (765), 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.

**e. Zinc (and Lead)**

Zinc status of astronauts, as assessed by serum zinc and urinary zinc excretion, did not change after long-duration spaceflight (31). Circulating zinc levels are an imperfect tool to evaluate zinc status, as other physiological factors may affect them (766). 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 (531, 545), and a similar increase in excretion of zinc was noted in Wistar rats flown during COSMOS 1129 (a 20-d spaceflight) (546). 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 (547, 548). 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 (767). Postflight data supported this model (767).

**f. Protein and amino acids**

Maintaining a proper protein intake is critical, as both low-protein and high-protein diets can cause harm (and, at the extreme, death). 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 (768), a fact confounded by the type of protein (and amino acids) consumed and by their relationship to other dietary factors (769, 770). The key to understanding the interrelationship of protein and bone may lie in understanding the complexities of the studies from which data are obtained, and understanding these complexities may require a full accounting of many nutrients and environmental factors (659, 746).

A low-protein diet (below the recommended dietary allowance) for up to 4 weeks can decrease calcium absorption and induce hyperparathyroidism in otherwise healthy subjects (85, 86). The impact of chronic low protein intake is not well understood; however, several studies suggest that low-protein diets are associated with loss of bone density (87, 88).

On the other hand, lowering excessive protein intake can have beneficial effects. High-protein diets lead to hypercalciuria, and increase the risk of fracture and the risk of renal stone formation (768, 771, 772). One 5-year study of 120 men revealed that the relative risk of stone formation due to a restricted protein (52 g/d) and salt (50 mEq/d) diet was half that due to a calcium-restricted diet (400 mg/d) (773). 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 the formation of calcium oxalate or calcium phosphate stones. One hypothesis to explain protein-induced hypercalciuria is related to the “acid-ash” hypothesis that excessive animal protein intake provides excess sulfur-containing amino acids that are metabolized to sulfuric acid. Because bone is a large reservoir of base, bone 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 (671).

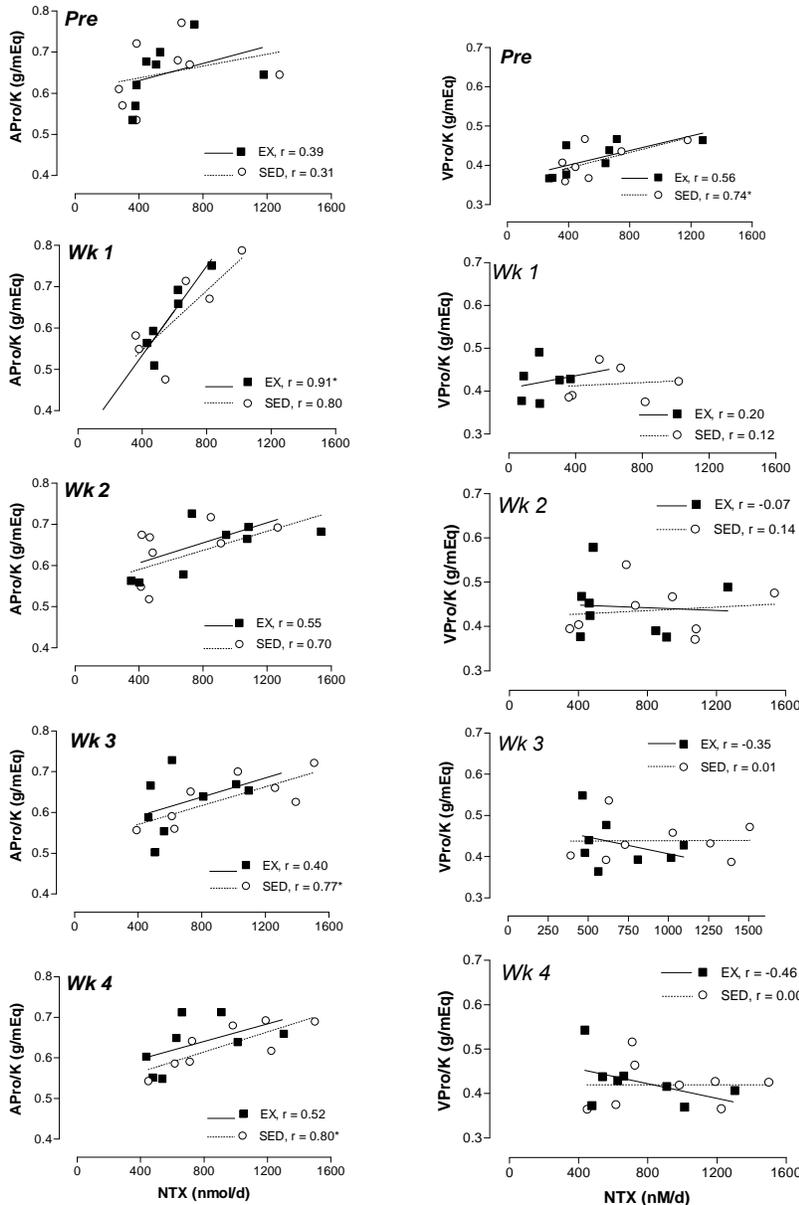


Figure 88. The bone resorption marker n-telopeptide (NTX) was positively correlated with the ratio of animal protein to potassium intake (APro/K) during weeks 3 and 4 of bed rest in sedentary (SED) bed rest subjects but only during week 1 in exercising (EX) bed rest subjects. The ratio of vegetable protein to potassium intake (Vpro/K) was correlated with urinary NTX excretion before bed rest but was not associated with NTX during bed rest (774).

Protein-induced hypercalciuria may be detrimental to bone as well as to risk of renal stones. Some studies show that high-protein diets increase calcium absorption (775), but this is currently not well accepted. Several studies show that animal protein increases acid load more than 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

that 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 of mainly vegetable protein (776). 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 (777). When the soy diet was supplemented with sulfur amino acids, urinary calcium and acid excretion increased. Conversely, the addition of dietary potassium (either as fruit or a K<sup>+</sup> supplement) to both diets decreased urinary calcium and acid excretion (777). 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 spaceflight) (774, 778).

Dietary intake of protein and specific types of protein, as well as patterns of acid and base precursors, have recently been associated with the concentration of urinary markers of bone resorption during bed rest (98, 691, 774). In one study, the relationships between acid and base precursors in the diet and markers of bone and calcium metabolism in male identical twins during bed rest were investigated (296). 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 the latter part of bed rest (Figure 88) shows the positive correlation between urinary NTX excretion and the ratio of animal protein to potassium intake during weeks 3 and 4 of bed rest. No relationship was found between the ratio of vegetable protein to potassium and markers of bone metabolism (Figure 88, right column). 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 (820).

The results from the twins study described above, showing that the ratio of animal protein to potassium intake was less related to bone metabolism markers in the exercising group and more related to bone markers at the end of bed rest when calcium excretion was highest, 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 (768, 770).

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 (778). The supplement contained 1.5 g of methionine, which is about 1.13 times the recommended daily intake, in addition to 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 89) (778). It was hypothesized (250) that this mild metabolic acidosis contributed to the higher urinary concentrations of bone resorption markers (Figure 90) and calcium excretion (Figure 91) in the supplemented group (778).

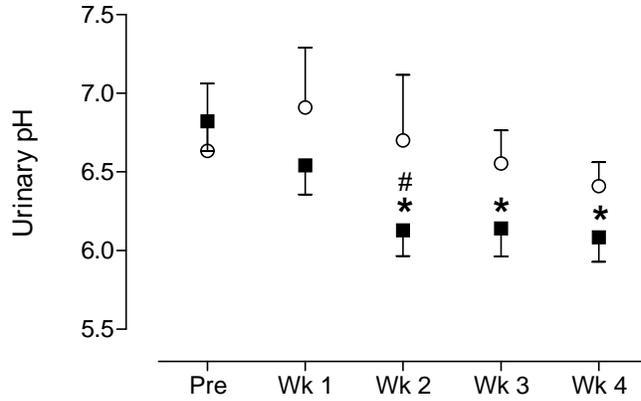


Figure 89. Urine pH (mean  $\pm$  SD) of amino acid-supplemented (AA, ■) and placebo (CON, ○) groups during 4 weeks of bed rest. \*Significantly different from pre-bed rest,  $P < 0.05$ . #Significant difference between groups,  $P < 0.05$  (778).

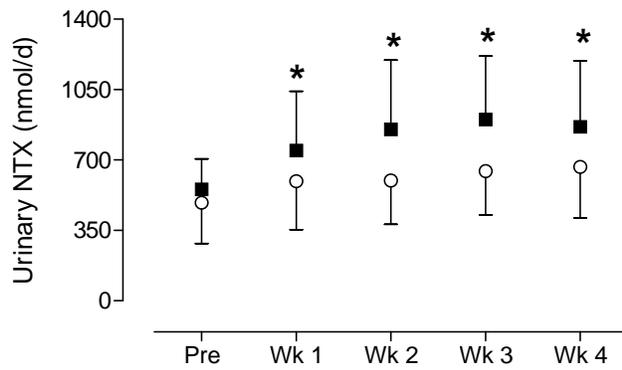


Figure 90. Urinary n-telopeptide (NTX) excretion (mean  $\pm$  SD) of amino acid-supplemented (AA, ■) and placebo (CON, ○) groups during 4 weeks of bed rest. \*Significantly different from pre-bed rest,  $P < 0.05$  (no significant difference between groups) (778).

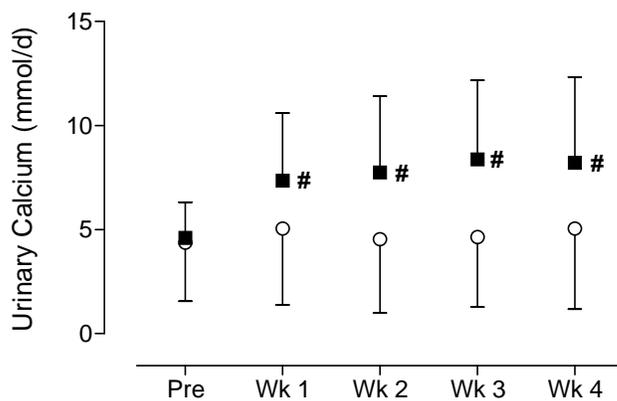
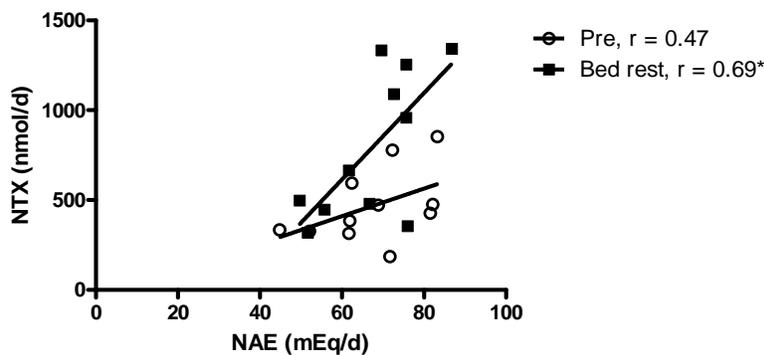


Figure 91. Urinary calcium excretion (mean  $\pm$  SD) of amino acid-supplemented (AA, ■) and placebo (CON, ○) groups during 4 weeks of bed rest. #AA values were significantly different from pre-bed rest,  $P < 0.05$  (778).

### Risk Factor of Inadequate Nutrition

In a separate study, 11 volunteers were subjected to 60-90 days of 6° head-down-tilt bed rest (357). 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) (Figure 92). Net acid excretion has also been associated with calcium loss using meta-analysis techniques (779).



**Figure 92.** Urinary n-telopeptide (NTX) excretion was positively correlated with net acid excretion (NAE) during but not before bed rest. NAE was estimated from the diet (357).

The current documented spaceflight requirement for protein intake is 0.8 g/kg per day, not to exceed 35% of the total daily energy intake (97). About 2/3 and 1/3 of the total amount of protein should be provided in the form of animal protein and vegetable protein, respectively. In the U.S., the recommended dietary allowances for those in the age range of the astronaut population are 56 g/d for men and 46 g/d for women (18). The acceptable distribution range for protein intake is 10% to 35% of the total energy intake (18). For historical reference, the spaceflight daily protein requirements were defined in 1991 for missions of 30 to 120 days as 10% to 15% of the total energy intake (4), and in 1995 for missions up to 360 days as 10% to 15% of the total energy intake (5). Actual intakes of protein typically exceed these recommendations, as discussed earlier.

## **5. Unique Aspects of Calcium and Spaceflight**

### **a. 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 (191). 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 (191). 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. Single-void calcium data from the in-flight data showed that neither of the suggestions looked promising for lowering the calcium concentration of urine that enters the UPA (191). With respect to bisphosphonate treatment, people sometimes have the impression that the pharmacological block of bone resorption will reduce urinary 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 (421).

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 water recovery from 70% to 74%, which allows a savings of >80 L H<sub>2</sub>O/year.

### **b. 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 spaceflight. 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 noncontrolled 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 (780). This technique is based on natural, biologically induced variations in the presence of the naturally occurring stable (nonradioactive) calcium isotopes ( $^{40}\text{Ca}$ ,  $^{42}\text{Ca}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ ,  $^{46}\text{Ca}$ , and  $^{48}\text{Ca}$ ), which react at different rates depending on their mass (781). These variations exist because bone formation favors the lighter isotopes, and thus the blood and urine (and other “soft tissues”) tend to contain 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 (780).

As the relationship between calcium isotopes and whole-body bone mineral balance is well established (782-784), 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  $0.25 \pm 0.07\%$  (1 SD) of their bone mass from day 7 to day 30 of bed rest (780). This rate of loss extrapolates to a loss of  $1.36 \pm 0.38\%$  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 (676).

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 spaceflight studies in which changes in bone formation and resorption are not only being altered by spaceflight itself but are being manipulated by various countermeasures.

## **B. Muscle and Protein**

### Muscle Loss

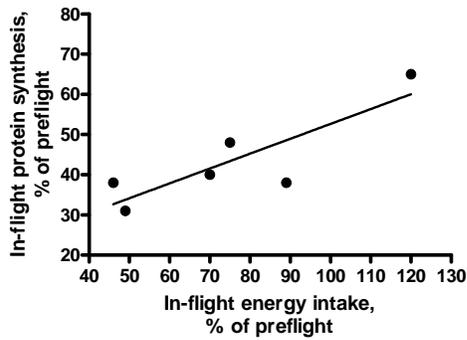
#### Spaceflight

Exposure to microgravity reduces muscle mass, volume, and performance, especially in the legs, on both short (38, 785-790) and long flights (38, 785, 791-795). Muscle biopsy studies demonstrated postflight decreases in cross-sectional area only in type II (fast-twitch) myofibers, the muscle fiber type that responds to resistive exercise (796).

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 spaceflight. As a result, the topic of spaceflight effects on muscle is very complex, and defining and understanding the big picture is difficult, to say the least. This topic has been reviewed (786, 797-801), and the difficulties of interpreting findings across the literature have also been noted (790, 797, 800).

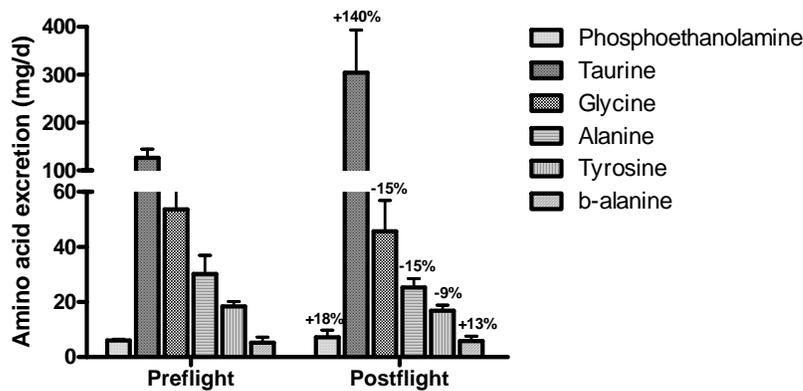
The amount of protein in the body or individual tissues is affected by the balance of protein synthesis and protein catabolism. Studies to understand changes in body protein, ideally, will include measurement of both of these factors in addition to turnover. Measuring these factors is not easy, and the results are variable (795, 802), 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 spaceflight, whole-body protein turnover and protein synthesis increase, and a greater percentage increase occurs in protein breakdown (96, 803). The increase in synthesis is hypothesized by Stein et al (801, 804) to be related to physiological stress, as indicated by generally (but not consistently) increased urinary cortisol during flight (25, 28, 130). These findings are similar to those found in catabolic patients—patients undergoing metabolic breakdown. Decreased prostaglandin secretion has also been implicated in the loss of muscle tissue during spaceflight, secondary to decreased mechanical stress on muscle (130). On long-duration Mir flights, conversely, investigators noted decreased rates of protein synthesis (22), secondary to reduced dietary energy intake (69) (Figure 93).



**Figure 93.** Protein synthesis and energy deficit. Adapted from Stein et al (69).

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 (805, 806). Limited Shuttle flight data indicate a tendency for plasma branched-chain amino acids to be increased during flight, relative to preflight levels (807). Data from short-duration Shuttle flights have revealed little or no change in urinary amino acid profiles (36), but data from the Apollo (Figure 94) and Skylab missions showed increases in urinary excretion of the amino acid metabolites creatinine, sarcosine, and 3-methylhistidine (434), suggesting that contractile proteins of skeletal muscle are degraded in weightlessness.



**Figure 94.** Urinary amino acid excretion by Apollo crewmembers (N=12) before and after flight (2, 104).

### 1. Ground Analog Studies

Ground-based rodent studies generally show increased proteolysis along with reduced protein synthesis (795). This pattern is similar to that seen in studies of humans during flight, described above.

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 (808). Untreated bed rest and ULLS both result in loss of muscle mass and strength, as nicely reviewed by Narici and de Boer (797).

All of these models provide a means to collect data that would be difficult if not impossible to collect during actual spaceflight, and data can be collected from 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 spaceflight.

Differences between flight and ground studies may relate to a number of variables, identifying potential shortcomings of the analog studies. 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 (696, 809-815). Evidence of increased protein catabolism during bed rest is limited, as reviewed by Bodine (800). There are 2 key confounding, and intertwined, issues (among others) with this area of research: energy intake and inflammation.

Energy consumption during spaceflight 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 IV.A, “Food and Energy.” On the Spacelab Life Sciences missions, in-flight intakes of protein and energy were about 20% less than preflight intakes, and crewmembers lost about 1% to 1.5% of their body mass (96). The prospect of comparing the controllable ground-analog model with actual spaceflight, in which underconsumption can occur, presents a study design issue. Ground-based studies typically have prescribed and controlled dietary intakes or are designed to maintain body mass. With respect to intake, investigators typically either seek to maintain energy intake of the subjects during bed rest, or restrict calories to recreate the hypocaloric intakes of spaceflight. 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 (24, 816), 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 spaceflight 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 (that is, these crewmembers were participating in metabolic studies and were required to consume the planned menu) (817, 818). 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 (41). This study is described in more detail elsewhere in the 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, the second key confounding issue in ground analog studies. Inflammation can lead to increased fat mass along with muscle loss (819-821). 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 (such as 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.

Inflammation is a key confounding issue in studies of muscle loss in spaceflight or ground analogs, and a common cause of inflammation is stress. Variability in stress levels may explain some of the variability in the results from studies of muscle loss, both flight and ground-based. 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 spaceflight. 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 inducing increased stress, 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 (822). 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 spaceflight (26, 823-825).

## 2. Muscle Loss Countermeasures

### a. Mechanical

Exercise is the most common first-pass approach to maintaining muscle mass and strength (786, 799, 826-828), but the exercise regimens tested as countermeasures to date have generally not succeeded in maintaining muscle mass or strength (or bone mass) during spaceflight (828). 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 (829). Similar findings (wide variations in exercise, lack of difference in bone loss) have also been documented for bone loss (63).

Many types of exercise protocols have been proposed to aid in the maintenance of both muscle and bone during flight (564, 683, 813, 830-839), 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 (79, 840); spaceflight 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 (714, 841-847). However, the efficacy, vibration dose, frequency, and duration of whole-body vibration exercise needs still further research. As mentioned earlier, mechanical stimulation is a prerequisite to avoid muscle and bone degradation. According to the mechanostat theory by Frost (848, 849), 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) (845). 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 (850). 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 (844, 847). 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 (844, 851-854).

**b. Pharmacological**

Testosterone has also been suggested as a muscle (and/or bone) loss countermeasure, on the basis of data from animal models (581, 727) and data documenting a reduction in testosterone concentrations during flight in humans, animals, and cellular models of spaceflight (728-734, 855). A potential confounding factor is the drop in testosterone that has been observed in exercising bed rest subjects (but not controls) (856).

The first in-flight testosterone data from human spaceflight 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 (857). 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 (733, 858). A significant confounding issue is that these crewmembers were consuming only about 60-85% of their basal metabolic energy requirements during the flight (9). Estimates of spaceflight energy requirements typically use an activity factor of 1.7 (that is,  $1.7 \times$  basal metabolic rate) (2). This factor is based on data documenting that total energy requirements are unchanged during flight (21), or in some cases are even increased with heavy exercise (22), 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 (9). 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) (859-861). Thus, the discrepancy between the long-duration data presented here 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 (Figure 95), apart from transient effects on landing day (583).

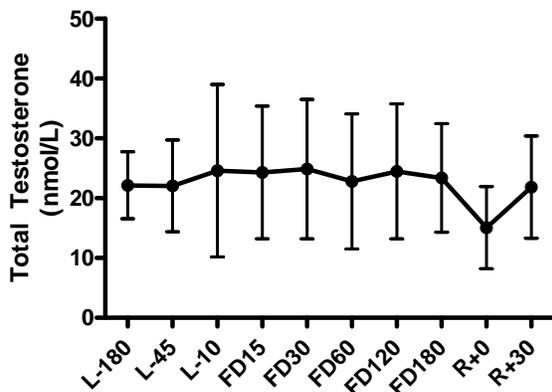


Figure 95. Total serum testosterone concentrations before, during, and after flight. Although circulating concentrations decreased significantly after flight (at R+0), no other time point differed significantly from the preflight mean. N=15. Data are from Smith et al (583).

The Skylab data include urinary testosterone from 3 crewmembers; at 2 in-flight data points, excretion was increased relative to the preflight period (583, 857). Plasma data from the 3 Skylab missions (N=9) are reported to have “showed a trend toward lower values after the mission” (857). Although we do not have urinary testosterone data on all crewmembers, these reports from the 1970s confirm the findings from ISS (583).

Bed rest analog data from a number of studies demonstrate that bed rest has no effect on circulating testosterone concentrations (582, 583, 812, 856, 862). Consistent decreases in serum testosterone were observed after the 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 (583).

Bed rest studies have generally shown no effect of bed rest on circulating total or free testosterone (582, 583, 812, 856, 862) 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 (356), 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 (583) (Figure 96).

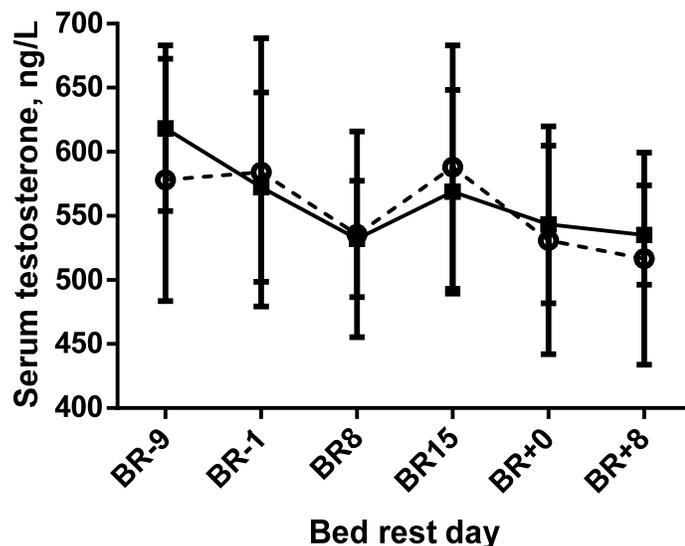


Figure 96. Total serum testosterone concentrations in subjects before, during, and after bed rest with (dashed line and open circles) or without (solid line and filled squares) an artificial gravity countermeasure. These data have been published (356), although the pre-bed rest data were combined as an average in the original manuscript. In this figure, the 2 pre-bed rest data points are graphed separately, as reported in (583).

One criticism of sedentary bed rest studies as an analog for spaceflight is that astronauts are not sedentary, especially on long-duration missions, when they exercise extensively. Wade et al reported that bed rest subjects with intensive exercise protocols had lower non-fasting circulating plasma testosterone concentrations than non-exercising bed rested controls (856). In this 4-week study they reported a small loss of (non-fasting) post-breakfast body mass (856, 863), 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 (863).

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 (864). 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 (865). 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 spaceflight.

As reviewed by Tou (866), in studies of rats with sample collections after spaceflight serum (867) and urinary (868) testosterone were generally decreased relative to the preflight period (869). 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 spaceflight (583). 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 (581, 727, 870). Unloading of longer duration (6 wk) in rats resulted in impaired spermatogenesis, but had no effect on circulating testosterone concentrations (871). Similarly, the production of testosterone by rat testes after actual spaceflight is diminished, as is response to stimulation by luteinizing hormone (LH) (872). 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 (873). 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 study interpretation. 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 (874), but with altered testicular physiology (875), including impaired Leydig cell responsiveness to LH 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 (582). Administration of testosterone in suspended rats mitigates muscle and bone losses (581). Although the bone data in rats are confounded by differential effects on growing and adult rats (876), 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 (877). That is, those with normal 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 (730), as well as in rats (868, 870). In a similar study, effects of hypergravity were found on tissues of rats and some endocrine variables, but increased gravitational force had no effect on circulating testosterone (867). 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) (730, 867). 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. There are reports of these treatments improving physical performance, muscle mass, and muscle strength in both young athletes and older sedentary men (878). The interaction of endocrine factors, aging (including middle age), the spaceflight environment, and the use of exercise to replace loading is not well understood.

In summary, 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 spaceflight on the human body.

### **c. Nutritional**

Use of protein and amino acid supplementation has long been studied as a potential means to mitigate muscle loss associated with spaceflight (79, 108, 825, 879-883), 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 (884), whereas feeding a bed rest group adequate energy with excess protein reversed nitrogen losses (814). 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 (94, 95, 792). In another bed rest study, a leucine-enriched, high-protein diet failed to mitigate muscle loss, and at some sites exacerbated loss (881). 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 (885), 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 (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  $\leq 0.8$  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, “Nutrients and Muscle Health,” 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.

### 3. Nutrients and Muscle Health

#### Protein

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. 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 (85, 886-888). 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.

It is our contention that studies of the effect of protein intake on muscle are often too short-term (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, of amino acid supplementation during bed rest, certainly gives reason for concern to 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 Spaceflight Evidence Book.

**XI. N7.2: We need to identify the most important nutritional factors for cardiovascular health.**

Cardiovascular issues are a key concern for space travelers (889-895), 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.

**A. Energy**

Cardiovascular deconditioning and loss of plasma volume are negatively correlated with energy consumption during bed rest (72) and after spaceflight (Figure 97). That is, insufficient energy intake is associated with greater plasma volume loss and cardiovascular deconditioning. The spaceflight 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, and these have also been included in recent nutrition reports (6).

Dr. Carpentier's data from astronauts in the early U.S. 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 97 and Figure 98 are based on Gemini and Apollo mission data. Combined with the data presented in Figure 99, these data clearly link energy intake and plasma volume loss with cardiovascular health during and after spaceflight.

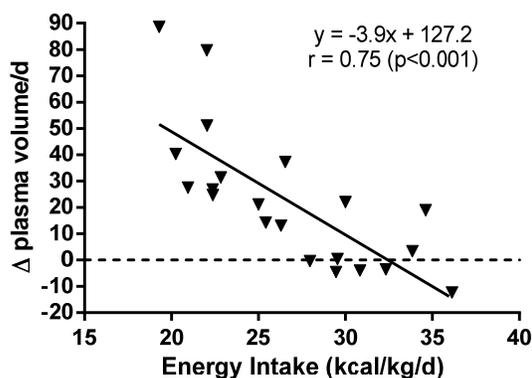


Figure 97. Relationship between energy intake (kcal/kg body mass/d) and plasma volume loss (mL/d) during Apollo missions. N=21. Data are courtesy of William Carpentier (6).

## Risk Factor of Inadequate Nutrition

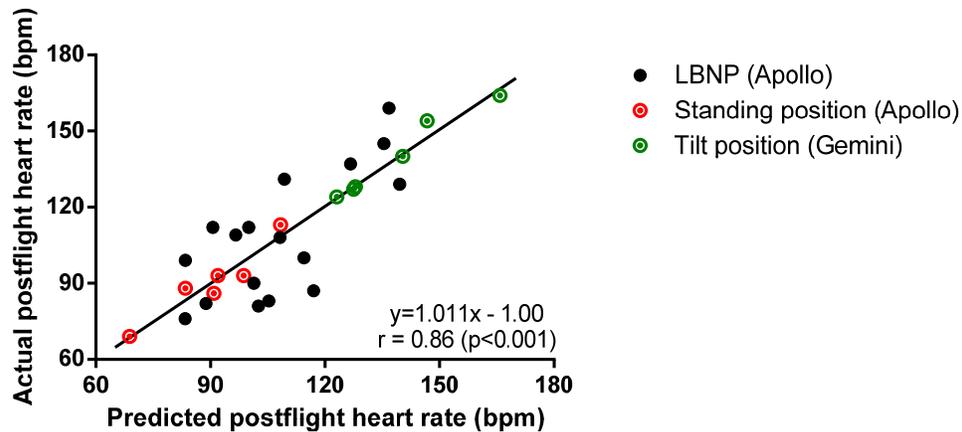


Figure 98. Postflight heart rate under 3 conditions, predicted (from factors including plasma volume loss) versus actual measured heart rate. These data are based on the following equations:

LBNP:  $29.00 - (0.064 \times \text{flight duration}) + (1.45 \times \text{resting preflight heart rate}) + (4.2553 \times \text{plasma volume loss, mL/h flight})$

Standing:  $77.23 - (0.52 \times \text{flight duration}) + (1.9098 \times \text{preflight resting heart rate}) + (17.4758 \times \text{plasma volume loss, mL/h flight})$

Tilt:  $330 - (2.53 \times \text{preflight resting heart rate}) - (7.20 \times \text{plasma volume loss, mL/h flight}) - (0.078 \times \text{flight duration, h})$

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 (6).

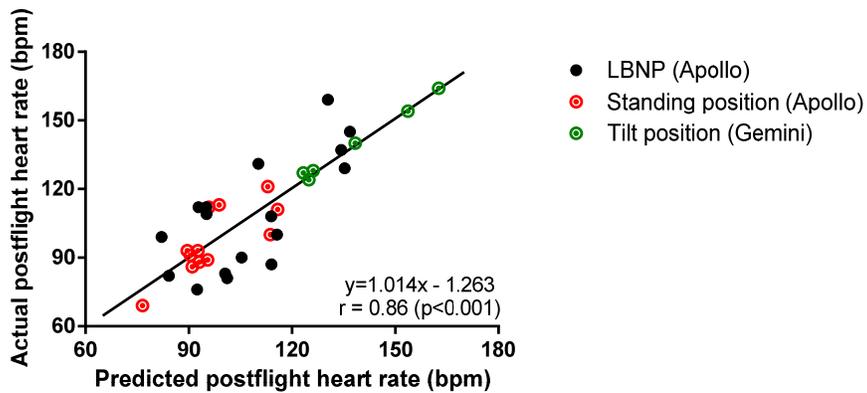


Figure 99. Postflight heart rate, predicted (from factors including energy intake) versus actual measured heart rate. These data are based on the following equations:

LBNP:  $63.02 + (1.18 \times \text{preflight resting heart rate}) + (0.48 \times \text{energy intake, kcal/d/kg}) - (0.17 \times \text{flight duration})$

Standing:  $115.87 + (1.12 \times \text{preflight resting heart rate}) - (2.17 \times \text{energy intake, kcal/d/kg}) - (0.17 \times \text{flight duration})$

Tilt:  $239.48 - (0.75 \times \text{preflight resting heart rate}) - (1.86 \times \text{energy intake, kcal/d/kg}) - (0.052 \times \text{flight duration})$

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 (70, 821). 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 spaceflight puzzle that is still missing is the effect of flight duration. That is, the data presented in Figure 97, Figure 98, and Figure 99 were generated from short-term flights, and this relationship may change on longer ISS missions. Given the inference from Figure 97 that energy intake should be greater than 33 kcal/kg body mass to avoid plasma volume loss, we evaluated ISS intake data (Figure 100) and found that few crewmembers are meeting this threshold.

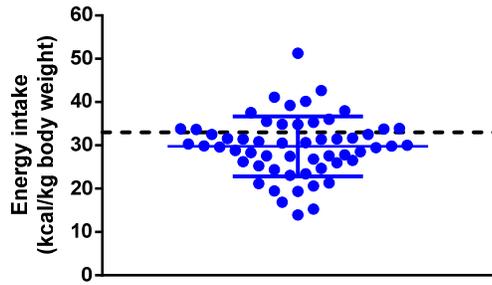


Figure 100. Energy intake during spaceflight on ISS missions (N=56). Each point represents an individual crewmember, and is their reported average daily energy intake over the course of the mission. Lines represent the mean  $\pm$  1SD (6).

## **B. 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 (896-898). Consistently decreased magnesium excretion after flight is a concern for many reasons (6), and warrants further investigation with respect to cardiovascular deconditioning during and after flight.

## **C. Ongoing and Future Research**

### **1. Oxidative Stress**

The role of oxidative stress and antioxidants in cardiovascular adaptation to spaceflight is currently being studied on ISS. Despite the facts that several oxidative stressors (eg, radiation, extravehicular activity, iron stores, exercise) could affect astronauts during flight, abundant evidence exists that oxidative stress occurs during spaceflight (reviewed later), and a wealth of literature exists about oxidative stress causing damage to the cardiovascular system, these three areas have never been evaluated. A study initiated on ISS in 2013 aims to accomplish the goal of 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.

### **2. Omega-3 Fatty Acids**

While omega-3 fatty acids have a clear beneficial impact on cardiovascular health on Earth, such effects have not been evaluated during 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.

### **3. *Healthier Diets***

Although it is easier to study individual nutrients in a controlled, experimental fashion, the effect of overall dietary quality is a topic that continues to gain ground, particularly as individual supplement studies fail to produce the “magic” supplement. Overall dietary quality, including fruit and vegetable intake, fish (omega-3 and vitamin D) intake, and food rich in phytochemicals and lower in sodium all combine to provide broad health effects (647, 748-750, 899, 900). Many of these converge on the cardiovascular system, along with benefits for bone, muscle, kidney, and other systems.

In 2014, NASA embarked on an effort to provide healthier food options for astronauts on ISS missions. As of this writing, the exact implementation of this effort is still in question, but the authors remain optimistic. While decreased bone loss is expected to be a primary outcome of the effort, it is clear that providing healthier food will have implications for many other physiological systems (eg, cardiovascular, muscle, immune) and other risks of spaceflight (e.g., radiation and oxidative stress), not to mention potential benefits for crew performance and morale.

One specific objective of this effort was to increase fruit and vegetable intake. Population-based and even smaller controlled studies have shown extensive benefits from inclusion in the diet of 5 or 6 servings of fruits and vegetables per day (737). Higher fruit and vegetable intakes have been associated with prevention of chronic diseases, including cardiovascular disease (748-750, 901) and cancer (748, 749).

The outcome of the effort to provide healthier foods for astronauts 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.

## **XII. N7.3: We need to identify the most important nutritional factors for ophthalmic health.**

### **A. Overview**

An initial ophthalmic concern for space travelers is cataract risk. While this risk is higher in astronauts (believed to be related to oxidative stress, as described earlier), benefits of dietary measures to prevent cataracts have been shown in the general population. Along with a recent attempt to fly healthier foods for astronauts, a key goal has been increasing fruit and vegetable intake by astronauts. Cataract risk has been shown to be lower in vegetarians (902), strengthening this call for more fruits and vegetables in the space diet. Vegetarianism is not the answer in and of itself, as nutrient risks are associated with this as well. With regard to bone, one study did not find a relationship between bone health and vegetarianism, and this was believed to be due to insufficient calcium intake in this group (903). Protein, iron, and vitamin B<sub>12</sub> are also common concerns for those following strict vegetarian diets.

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 (271, 272, 274), some crewmembers have experienced vision-related changes after long-duration spaceflight. These changes include optic disc edema, globe flattening, hyperopic shifts, choroidal folds, and cotton wool spots (560). 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<sub>12</sub>-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 spaceflight.

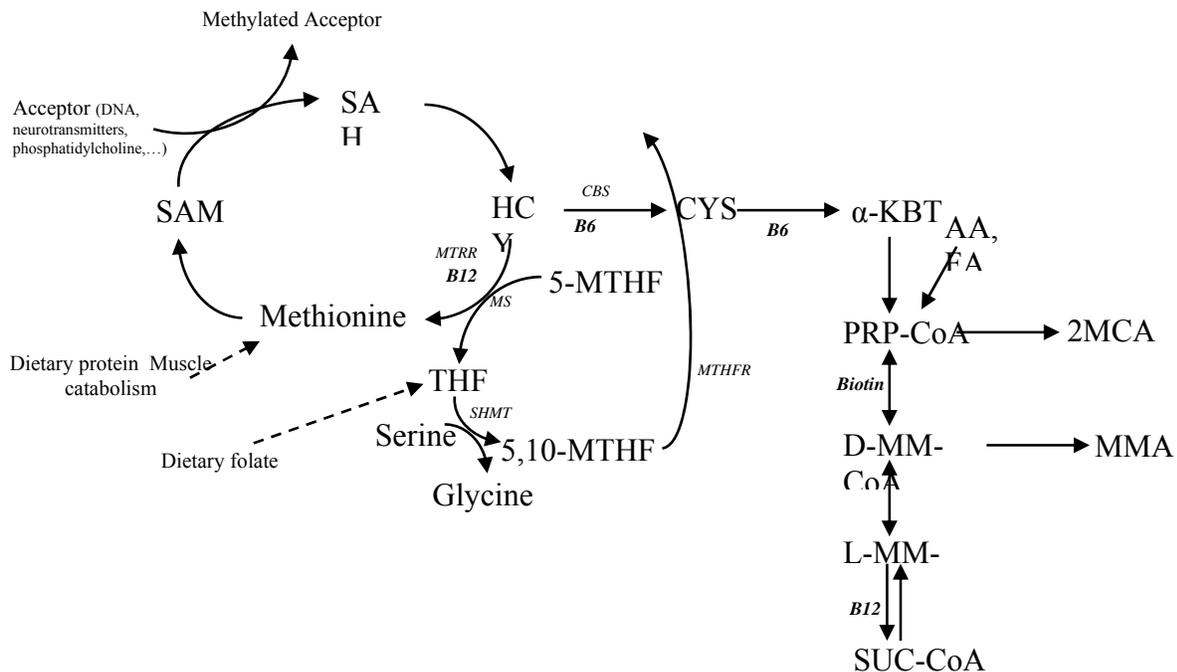
### **B. Ophthalmic Changes**

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 (560). Since that first report was published, 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 (904). The mechanism of the ophthalmic changes is not known, but microgravity-induced fluid shifts (2, 158), elevated exposure to CO<sub>2</sub> in the spacecraft cabin, possible intraocular pressure or intracranial pressure changes, and local intraorbital (choroidal 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<sub>12</sub>-dependent 1-carbon transfer pathway may be involved could help explain individual susceptibility to these ophthalmic changes.

### C. One-Carbon Metabolism

One-carbon metabolism refers to a pathway that requires folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>, and is involved in purine and pyrimidine synthesis, amino acid metabolism, and synthesis of the methylating agent S-adenosylmethionine (SAM, Figure 101). 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<sub>6</sub>, and vitamin B<sub>12</sub> can influence plasma homocysteine concentration (905, 906).

Much of the homocysteine literature has focused on its association with coronary artery disease, stroke, and migraines (905, 907, 908), but some studies show associations of homocysteine with ophthalmic health issues. Ophthalmic issues such as age-related macular degeneration result from lipid deposits under the retinal pigment epithelium (909) and decreased retinal vessel functionality (910), and some theorize that age-related macular degeneration is similar to the development of cardiovascular disease (911). 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 (389-391, 912). For instance, a meta-analysis showed that elevated plasma homocysteine was associated with an increased risk of primary open-angle glaucoma (913). Other meta-analyses have shown that increased serum homocysteine and low vitamin B<sub>12</sub> status were independently associated with increased risk for age-related macular degeneration (391, 914). Daily supplementation with folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> is associated with a 30% to 40% decreased risk for age-related macular degeneration (915).



## *Risk Factor of Inadequate Nutrition*

Figure 101. Overview of 1-carbon metabolism. AA, amino acids; CBS, cystathionine  $\beta$ -synthase; CYS, cystathionine; FA, fatty acids; HCY, homocysteine;  $\alpha$ KBT,  $\alpha$ -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

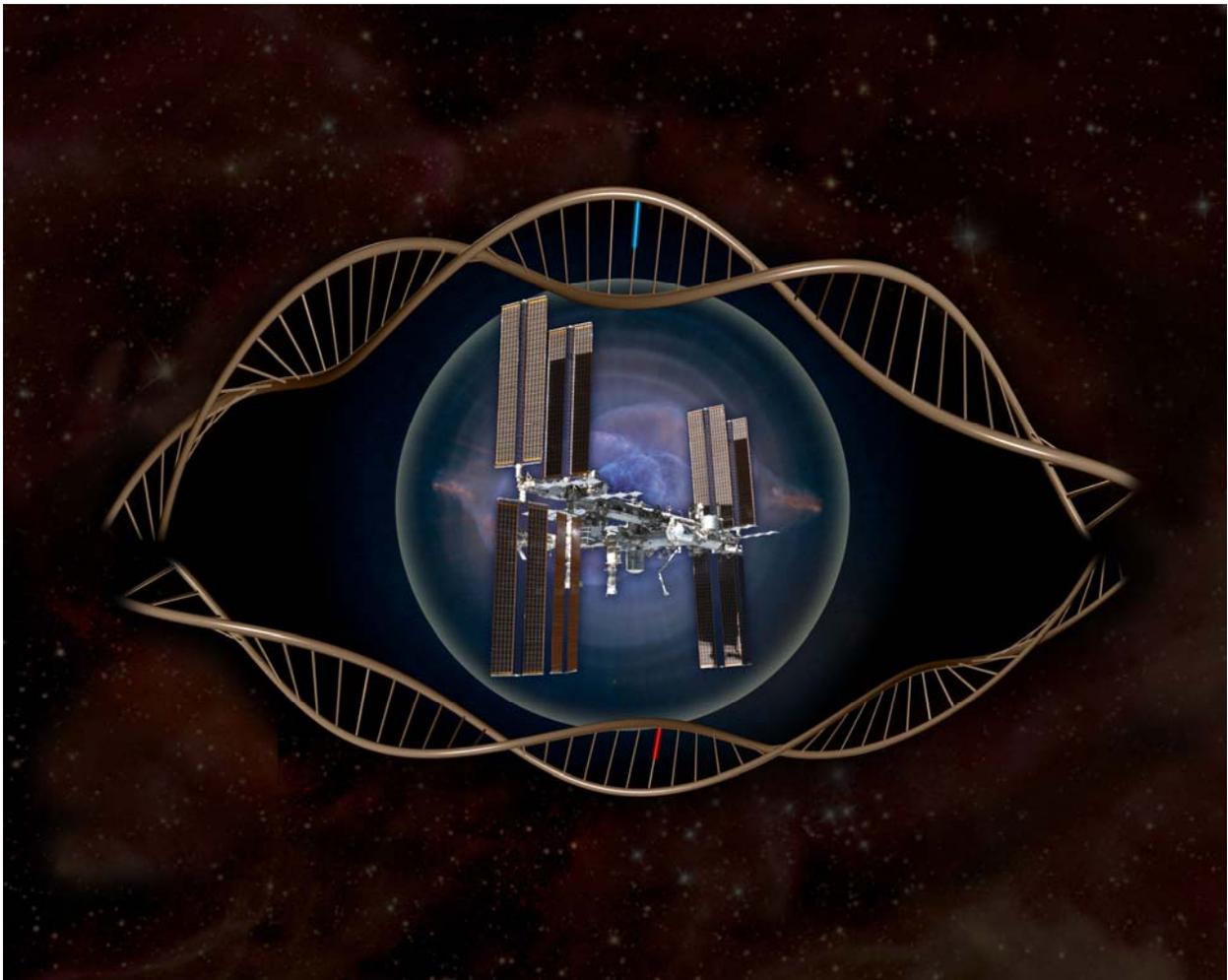


Figure 102. Artistic depiction of the vision issues related to spaceflight, and the potential for genetic influences on the understanding of this problem. The depiction of a different DNA base pair in the 2 strands reflects genetic polymorphisms, or multiple forms, of the DNA. Inset imagery includes 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 for exploration-class missions as well.

### **XIII. N7.4: We need to identify the most important nutritional factors for behavior and performance.**

*Note: Alexandra Whitmire of the Behavioral Health and Performance Element of the Human Research Program contributed to this section, and we greatly appreciate her help.*

#### **A. Overview**

The Behavioral Health and Performance Element (BHP) is one of the 6 elements in the NASA Human Research Program. BHP manages and implements a research portfolio to characterize and mitigate 3 specific risks for exploration-class missions and, in some instances, current Flight Medical Operations. Two of these risks—the risk of performance decrements and adverse health outcomes resulting from sleep loss, circadian desynchronization, and work overload, and the risk of adverse behavioral conditions and psychiatric disorders—are relevant to the question of nutrition of the crew during spaceflight. The primary outcomes of interest to BHP that are addressed here are fatigue, cognition, and mood and morale.

Although it is one of the more difficult areas to quantify, the interrelationship of food and nutrition with crew behavior and performance is undeniable. Anecdotal evidence from long-duration stays in extreme environments (such as Antarctica) indicates that as mission durations increase, food will play a stronger role in maintaining mood and morale. Non-space studies have reported on the relationships between dietary choices and psychological well-being, but this literature is relatively sparse as well, as nicely reviewed by Blanchflower et al (916). It is worth noting that psychological well-being is not just the absence of the negative, but also the presence of positive characteristics. These can include a sense of autonomy, competence and mastery, positive relations, and satisfaction with life and work. From our perspective, food and nutrition can have either positive or negative effects on psychological well-being. Furthermore, a comprehensive review of the role of specific nutrients in cognition was published by Gomez-Pinilla (917), and several examples from that review are highlighted herein.

#### **1. Energy**

Inadequate energy intake is associated with fatigue and other indices of behavior and performance. Ramadan fasting, during which food is not consumed for about 13 hours each day for 30 days, resulted in altered sleep patterns, perceived fatigue, and reduced exercise performance, along with metabolic, endocrine, and inflammatory alterations (918). These effects occurred despite the fact that energy intake during Ramadan was estimated to be more than 90% of pre-Ramadan intakes ( $2385 \pm 134$  kcal/d on day 21, compared to  $2596 \pm 133$  kcal/d on day 0) (918).

Biochemical evidence helps link energy intake with brain function. Signaling molecules in the brain link energy metabolism to synaptic processes, and one such molecule is brain-derived neurotrophic factor (BDNF) (Figure 103). It is found in the hippocampus and hypothalamus. BDNF has been shown to influence many aspects of energy metabolism, including appetite suppression, insulin sensitivity, and glucose and lipid metabolism (917).

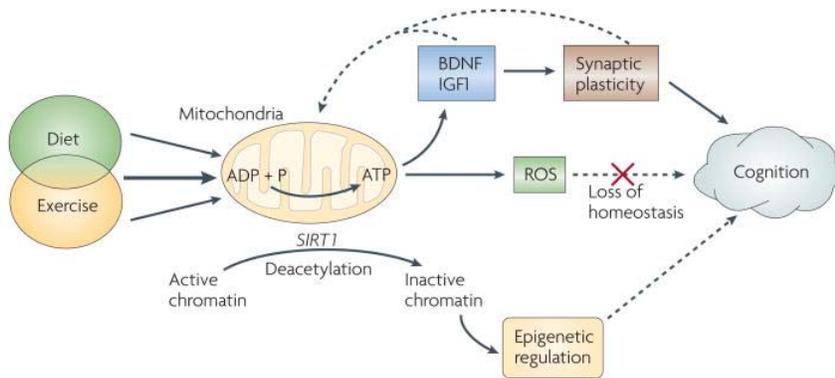


Figure 103. This figure shows how diet and exercise can ultimately affect cognitive function through BDNF, a signaling molecule that can alter synaptic plasticity. (Figure is from (917).)

In addition to energy consumption, meal times and nutrient content can also affect performance, cognitive function, and mood and morale (919-921). Evidence indicates that meal timing in particular can affect the phase of the circadian rhythm, and changes in circadian phase can moderate the food and performance relationship. Experimental evidence has now emerged on the location of at least one food-entrainable oscillator—there may be several—and suggests that the oscillator “gene” may be “induced” by fasting and refeeding (922).

## 2. Fruits and Vegetables

Recent attempts to improve provision of healthier foods for ISS crews had the objective of in-flight consumption of 6 servings of fruits and vegetables per day. A study with this objective was predicated on mitigating bone loss, and this specific aim was based on results from several studies showing that greater fruit and vegetable intake is associated with higher bone mineral density (656, 738, 740, 745, 923).

While greater fruit and vegetable intake of the general population has been advocated for some time now, the premise behind this advocacy has typically been linked to physical health as opposed to psychological well-being (916). Nonetheless, epidemiological studies have established a somewhat tenuous link between psychological well-being and fruit and vegetable intake, as reviewed by Rooney (924). Two recent large (close to 15,000 participants in each) surveys in the UK found positive associations between fruit and vegetable intake and well-being (925, 926). Others have shown that diets rich in vegetables, fruit, meat, fish, and whole grains are associated with lower odds for major depression or dysthymia, whereas “western” diets rich in sugar and processed foods were more likely to be associated with behavioral disorders (927). Supporting that concept are the results of animal studies that evaluated effects of “junk food” high in saturated fat and sugar; researchers found that after only 3 weeks on this type of diet, animals showed a decline in cognitive performance and reduced hippocampal levels of synaptic plasticity related to BDNF (928).

Anecdotal evidence from spaceflight demonstrates the salience of food to crewmembers during their stay on the ISS. Stuster provides an analysis of 10 astronaut journals maintained during their 4- to 6-month missions in space (929). “Food” was the tenth most commonly “journalled” category. Within this category, references to the onboard supply of meal items composed the most frequently reported subcategory, and most references in this subcategory

were written in response to running low on supplies during a few of the increments. As noted by Stuster, all of the participants described disappointment over the depletion of favored food and drink items, and many wrote about the joy of finding an item that had been believed to be gone. Relevant testimonials from Stuster (929) include these:

- *We are getting tired of eating chicken all the time, but it will be OK.*
- *The good news is that we have good food on board now. It makes such a huge difference in mental attitude. We should be fit and ready for the EVA.*
- *We actually have a pretty wide assortment of food and it's not bad at all. One of my concerns was being able to put up with the food for six months. Although it's not quite home cooking, the folks in the food laboratories in Houston and in Moscow have done a pretty good job on the menu, providing us a wide assortment of quality food and drinks.*
- *I really do wish for more American type breakfast food. The Russians eat a lot of cheese stuff, some of it quite good. I enjoy most of their food and am even trying new things that I didn't care for previously. I hope it doesn't get too "old" after several months!*
- *I discovered extra main course rations in my bonus containers, so we enjoyed a dinner of \_\_\_\_ and were very happy. It is amazing how this seemingly small thing made our evening. On the ground, this food would not be considered particularly appetizing. However, take it away from us and it seems like the most delicious food in the world.*
- *The food is getting somewhat old to us. On paper we have quite a variety and it is not too bad actually, but I think it is starting to all look and taste the same. Perhaps we are just tired of eating out of a can or wrapper.*

### **3. Specific Nutrients**

In several studies, investigators have evaluated interrelationships between nutrients and aspects of behavior and performance. These are briefly summarized in Table 1, which is adapted (and expanded) from Gomez-Panilla (917).

*Risk Factor of Inadequate Nutrition*

Table 1 Select nutrients that affect cognitive function (adapted from (917)).

<b>Nutrient</b>	<b>Effects on cognitive function and psychological health</b>	<b>Food sources</b>
Omega-3 fatty acids	Increased intake associated with improved cognitive performance in healthy adults (930). Amelioration of cognitive decline in the elderly (931); basis for treatment in patients with mood disorders (932); improvement of cognition in traumatic brain injury in rodents (933); amelioration of cognitive decay in mouse model of Alzheimer's disease (934, 935). More controlled studies required to help resolve inconsistencies in the literature (936).	Fish (salmon), flax seeds, krill, chia, kiwi fruit, butternuts, walnuts
Curcumin	Amelioration of cognitive decay in mouse model of Alzheimer's disease (937); amelioration of cognitive decay in traumatic brain injury in rodents (938).	Turmeric (curry spice)
Flavonoids	Cognitive enhancement in combination with exercise in rodents (939); improvement of cognitive function in the elderly (940).	Cocoa, green tea, ginkgo tree, citrus fruits, wine (higher in red wine), dark chocolate
Saturated fat	Promotion of cognitive decline in adult rodents (928); aggravation of cognitive impairment after brain trauma in rodents (941); exacerbation of cognitive decline in aging humans (942).	Butter, ghee, suet, lard, coconut oil, cottonseed oil, palm kernel oil, dairy products (cream, cheese), meat
B vitamins	B <sub>6</sub> and B <sub>12</sub> status related to increased incidence of depression and impaired cognition (943). Supplementation with vitamin B <sub>6</sub> , vitamin B <sub>12</sub> , or folate has positive effects on memory performance in women of various ages (944, 945); vitamin B <sub>12</sub> improves cognitive impairment in rats fed a choline-deficient diet (946). Combined B-vitamin (folate, B <sub>12</sub> , B <sub>6</sub> ) supplementation enhances cognitive performance (947) and memory (944, 948). Nonetheless, more controlled studies required to help resolve inconsistencies in the literature (949).	Various natural sources. Vitamin B <sub>12</sub> is not available from plant products
Vitamin D	Important for preserving cognition in the elderly (950), although more controlled studies required to help resolve inconsistencies in the literature (951) (952).	Fish liver, fatty fish, mushrooms, fortified products, milk, soy milk, cereal grains

*Risk Factor of Inadequate Nutrition*

<b>Nutrient</b>	<b>Effects on cognitive function and psychological health</b>	<b>Food sources</b>
Vitamin E	Amelioration of cognitive impairment after brain trauma in rodents (953); reduction of cognitive decay in the elderly (954).	Asparagus, avocado, nuts, peanuts, olives, red palm oil, seeds, spinach, vegetable oils, wheat germ
Choline	Reduction of seizure-induced memory impairment in rodents (955); a review of the literature reveals evidence for a causal relationship between dietary choline and cognition in humans and rats (956).	Egg yolks, soy, beef, chicken, veal, turkey liver, lettuce
Combination of vitamins (C, E, carotene)	Antioxidant vitamin intake delays cognitive decline in the elderly (957).	Vitamin C: citrus fruits, several plants and vegetables, calf and beef liver. Vitamin E: see above
Calcium, zinc, selenium	High serum calcium is associated with faster cognitive decline in the elderly (958); reduction of zinc in diet helps to reduce cognitive decay in the elderly; lifelong low selenium level associated with lower cognitive function and depressive symptoms in humans (959, 960).	Calcium: milk, coral. Zinc: oysters, a small amount in beans, nuts, almonds, whole grains, sunflower seeds. Selenium: nuts, cereals, meat, fish, eggs
Copper	Cognitive decline in patients with Alzheimer's disease correlates with low plasma concentrations of copper (961).	Oysters, beef/lamb liver, Brazil nuts, blackstrap molasses, cocoa, black pepper
Iron	Iron treatment normalizes cognitive function in young women (962).	Red meat, fish, poultry, lentils, beans
Glycemic load	Consumption of a high glycemic diet is associated with lower cognitive performance (963).	Sugar, processed grains

**B. Summary**

Although a fair amount of literature is available that pertains to food and nutrient effects on aspects of cognition, behavioral health, and performance, virtually none of it pertains to spaceflight. This is clearly an area where additional research is needed. Also, more integrative efforts are needed, in research on the ground and in spaceflight, to better understand the effects on food, hormones, stress response, sleep loss/circadian desynchrony, and exercise on behavioral, immunologic, cardiovascular, reproductive health, skeletal/muscle, and on sensorimotor responses.

#### **XIV.N7.5: We need to identify the most important nutritional factors for immune health**

##### **A. Overview**

Optimal function of the immune system is impaired in the presence of malnutrition (964). Without adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response (965, 966). Nutrients act as antioxidants and as cofactors (967). Historically, during spaceflight 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 (968-971). Such a lack of nutrients also leads to deregulation of the balanced host response (972). Disruption of nutritional balance and dietary intake of astronauts and cosmonauts during spaceflight, which is often accompanied by a stress response, might influence their immune response (973, 974). However, detailed information on the effects of many micronutrients during spaceflight, especially their relationship to immune system function, is mandatory before specific nutritional recommendations can be made.

We review in this section nutrients and their relationship to immune system function, even though some have been reviewed in detail earlier, and others will follow.

##### **B. 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, 326). On the Mir station and the Life and Microgravity Science Shuttle mission, space travelers consumed inadequate energy intake and had significant increases in urinary excretion of 8-iso-prostaglandin-F<sub>2</sub> $\alpha$  and 8-oxo-7,8-dihydro-2 deoxyguanosine (8-OH-dG), markers for oxidative damage (327).

##### **C. Protein and Amino Acids**

Stein et al suggest that spaceflight triggers a stress response similar to injury-induced stress (975). Protein and amino acid deficiencies can have profound effects on a variety of immune system functions (976, 977). With respect to specific amino acids, arginine is necessary for normal T-cell function and may become essential in catabolic states. In animal studies, supplementary dietary arginine has been shown to have useful effects on cellular immunity, including 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 (978). Supplementation of arginine led to improved wound healing and immune responses in elderly subjects (979). 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 spaceflight.

Another amino acid beneficial for the immune system is glutamine. Glutamine is the most

abundant free amino acid in the body. It can inhibit activation of the NF- $\kappa$ B nuclear transcription factor and cytokine expression after sepsis (980). 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 a stimulator of nucleotide synthesis (981, 982). Glutamine seems to have a significant beneficial effect on mortality, length of hospital stay, and infectious morbidity in critical illness (981). Positive results of glutamine supplementation have been shown in critically ill patients in whom supplemental glutamine reduced complications and mortality rates as well as stimulating actions on the immune system (983, 984). However, up to now supplementation of glutamine as a pharmaconutrient has not been tested in spaceflight or spaceflight analogs such as bed rest.

#### **D. Vitamin D**

As already described in previous chapters, the classical function of vitamin D is to regulate calcium homeostasis and thus bone formation and resorption. However, recent publications show that vitamin D also has 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 (985). 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)<sub>2</sub>D<sub>3</sub> in the immune system (986). Even the enzyme 25(OH)D<sub>3</sub>-1- $\alpha$ -hydroxylase is expressed by active macrophages, making them able to synthesize and secrete 1,25(OH)<sub>2</sub>D<sub>3</sub> (987). However, in macrophages the enzyme is mainly activated by immune signals such as interferon (IFN)- $\gamma$  rather than by parathyroid hormone, which is the activator in the kidney (985). Moreover, the active vitamin D metabolite 1,25(OH)<sub>2</sub>D<sub>3</sub> 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)<sub>2</sub>D<sub>3</sub> suppressed both bacillus Calmette-Guérin and *Mycobacterium tuberculosis* in infected cell cultures, likely through “nonclassical” mechanisms including the induction of antimicrobial peptides (988, 989). Kondo et al found that vitamin D supplementation improved the sensitivity of the treatment response to pegylated interferon  $\alpha$ /ribavirin therapy in chronic hepatitis C patients (990).

Studies during or after spaceflight 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 (991). 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 (312). 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  $\mu$ g/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  $\mu$ g/dL or below, Figure 104). 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.

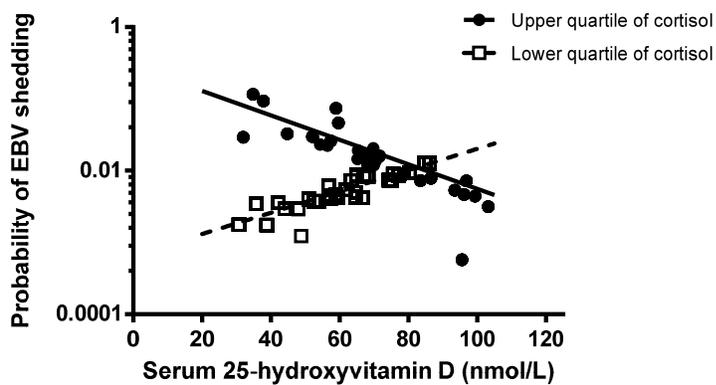


Figure 104. An interaction between serum cortisol, vitamin D status, and the probability of EBV shedding. Data from all 41 participants in the Antarctic study are included in the graph, and the data were statistically analyzed using the continuous data set of cortisol data. The data are split into the 2 subgroups here for presentation purposes. The graph is from Zwart et al (312).

#### E. Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> functions in many enzymatic reactions, and deficiencies result in anemia, as well as neurological disorders. Vitamin B<sub>12</sub> functions as a coenzyme in 2 metabolic forms: adenosylcobalamin and methylcobalamin. Vitamin B<sub>12</sub> 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 β-leucine. Vitamin B<sub>12</sub> deficiency may cause the accumulation of folate in the serum because of a reduction in B<sub>12</sub>-dependent methyltransferase, also known as the methyl-folate trap (386). Vitamin B<sub>12</sub> also functions in the synthesis of choline, which can be converted to the neurotransmitter acetylcholine. Vitamin B<sub>12</sub> deficiency may lead to alterations of immunological indicators, such as a reduction of lymphocytes and suppressed natural killer (NK) cell activity, both of which can be reversed by supplementation with vitamin B<sub>12</sub> (992). In one study, for 4 months elderly subjects (aged 70 years) received, in addition to their regular diet, a special nutritional formula providing, among other nutrients, 120 IU vitamin E, 3.8 mg vitamin B<sub>12</sub>, and 400 mg folic acid. NK-cell cytotoxic activity increased in supplemented subjects, indicating increased innate immunity in elderly people (993). These few studies demonstrate the importance of a sufficient B-vitamin status to maintain an adequate immune response (994).

#### F. Sodium

High sodium intake is correlated with development of hypertension in sodium-sensitive people. We have shown in spaceflight 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 (40, 173, 995). A hypothesis that might explain how sodium can be bound in an osmotically inactive way has been brought forward by Titze et al (206, 996) 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 (201, 202, 997). 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.

#### **G. Vitamin A**

In brief, vitamin A plays a well-known role in immune function and protection against infections (998-1001). A vitamin A deficiency impairs mucosal barriers and diminishes the function of neutrophils, macrophages, and natural killer cells (1002); it may affect host defenses directly (1003) or indirectly through its role in epithelial cell differentiation and host barrier function (1000). 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 who have minimal or poor vitamin A status. However, whether immunity benefits accrue from providing additional vitamin A to those with sufficient status is not known (1004).

#### **H. 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 reactions and of metabolic checkpoints controlling activation and survival of immune cells (972). A deficiency in vitamin C status is associated with reduced immune function (1005). Vitamin C has been shown to stimulate the immune system by enhancing T-lymphocyte proliferation in response to infection, and by increasing cytokine production and synthesis of immunoglobulins (1006). However, the antioxidant role during spaceflight 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 (357, 667), but a trend for an increase was apparent. This might have been related to dietary vitamin C intake during the study relative to the intake before the study (357).

#### **I. Vitamin E**

Vitamin E is a strong antioxidant that can support monocyte/macrophage-mediated responses (1007). Vitamin E and selenium have synergistic functions in tissues to reduce damage to lipid membranes by the formation of reactive oxygen species 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 (that is, able to be oxidized) form. Additionally, vitamin E may improve T-cell function by decreasing production of prostaglandin E<sub>2</sub> by macrophages, by modulating the amino acid cascade initiated by lipoxygenase and/or cyclooxygenase (131). 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.

**J. Copper**

Copper has wide-ranging functions in the body, including many considered vital for spaceflight (102, 533-536). 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 spaceflight.

**K. Zinc**

In addition to its many essential functions in growth and development, zinc is essential for the function of cells of the immune system (1008). 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 spaceflight (2). No data are available on the use of zinc supplementation as a countermeasure during spaceflight.

**L. Polyphenols**

Naturally occurring polyphenols like resveratrol, quercetin, curcumin, and catechins have shown antioxidant and anti-inflammatory effects (1009). These effects seem to be modulated through different pathways such as protein kinase-dependent pathways activated by the nuclear transcription factor NF- $\kappa$ B or by mitogens, as well as through preventing the generation of reactive oxygen species by iron binding (1010). Additionally, polyphenols seem to activate sirtuin 1 directly or indirectly and are beneficial for that reason, besides their other functions in 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 (537, 1011-1016). 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 spaceflight. 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 spaceflight yet; however, a recently selected flight study will examine the effect of increasing polyphenol intake on bone health in particular.

**M. Iron**

As mentioned previously, the maintenance of iron homeostasis is extremely important for human health. During spaceflight, it is well established that iron homeostasis is altered (2, 498). The decreased red blood cell mass, increased serum ferritin, decreased transferrin receptors, and increased serum iron all provide evidence for increased iron storage during spaceflight. Furthermore, the space food system provides almost 3 times the recommended iron 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 (1017, 1018). 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 (1019, 1020). Studies need to be done to determine the role of increased iron stores during spaceflight on immune function and reactivation of latent viruses.

#### **N. Polyunsaturated Fatty Acids**

Polyunsaturated fatty acids (PUFAs), such as omega-3 fatty acids, protect cells from oxidative damage and radiation-related risks (123, 1021), both of which are concerns for space travelers. The mechanism of action of omega-3 fatty acids in protecting cells is likely related to multiple pathways, but evidence exists that the nuclear transcription factor NF- $\kappa$ B is affected differently by omega-3 and omega-6 fatty acids (1022). This transcription factor affects transcription of genes involved in cell cycle regulation and inflammatory processes. NF- $\kappa$ B is activated by arachidonic acid and specifically by prostaglandin E<sub>2</sub>, but Camandola and colleagues have found that eicosapentaenoic acid (a PUFA) inhibits NF- $\kappa$ B activation (1022).

We have reported elevated NF- $\kappa$ B after short-duration spaceflight (147). The effects of omega-3 fatty acids on inflammatory cytokines, and specifically TNF $\alpha$ , are well documented on the ground (147, 1023-1025), but warrant further studies during spaceflight.

#### **O. Food, Microbiome, and Immunity**

Growing evidence documents the interrelationship of diet, the microbiome, immune system function, and disease (650, 1026-1030). This is a relatively new field, and while NASA has initiated some experiments evaluating the microbiome in ISS crews, more work clearly is required. Additional proposals have been submitted (and as of this writing are still under review) to evaluate the potential for developing and flying healthier foods and their ability to influence immune system function and other factors in health and disease.

#### **P. Summary**

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 spaceflight 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.

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To reach that level of individualized diet for astronauts, further research is needed to assess whether diet can benefit immune function during spaceflight. The use of basic clinical pharmacology, molecular biology, and clinical research principles in the study of nutritional therapy during spaceflight 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.

**XV. N13: Can renal stone risk be decreased using nutritional countermeasures?**

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. Generally, renal stone risk is elevated during spaceflight (181). As with any spaceflight effect, some crewmembers are more affected than others. The graph below (Figure 105) is an important illustration of this. Some crewmembers had very high elevations in brushite or calcium oxalate supersaturation during spaceflight, whereas others had very low levels of supersaturation. During spaceflight, environmental and dietary factors all greatly affect renal stone risk. Crewmembers who have an increased risk before spaceflight 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 spaceflight.

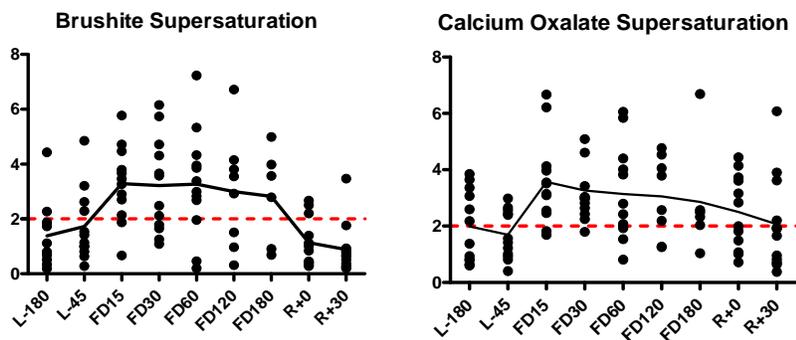


Figure 105. Estimated supersaturation risk from the Renal Stone Risk Profile before, during, and after spaceflight. Each symbol represents a 24-h urine pool, and the solid black line represents the group mean. The red dashed line is the point above which the risk is greater than in the non-stone-forming population (2, 6).

As of 2013, 25 renal stone events had 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 (765), 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 ISS for use at flight surgeon discretion if clinically indicated. The use of KCit was shown to increase urinary pH, thus 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 the risk of forming calcium-containing renal stones. 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 106).

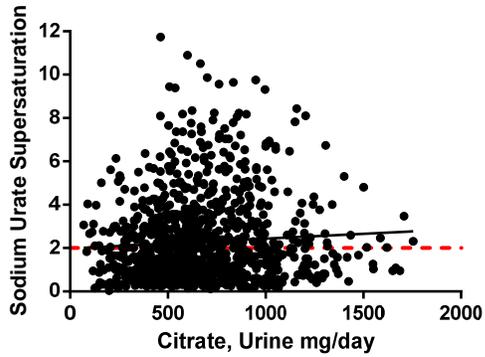


Figure 106. Relationship between urinary citrate and sodium urate supersaturation. The dashed line at 2 represents the average risk of sodium urate stone formation for the general population, with numbers above 2 being higher than average risk. Citrate, which typically protects against renal stone risk, is actually positively correlated with sodium urate supersaturation (Pearson  $r = 0.065$ ;  $P < 0.016$ ). Data are from 1399 24-h urine collections (2, 6).

## **XVI. Conclusion**

Nutrition is critical for health, both on Earth and in space. Determining the nutritional requirements for travelers on short-, medium-, and long-duration exploration missions is critical for ensuring crew health. Requirements have been defined, as described herein, but in most cases have never been validated. In other cases, requirements for specific foods have not yet been defined (e.g., fish, fruits, vegetables, nuts), and these play as critical a role as the defined nutrients, if not a larger one, given all we do not know about the thousands of phytonutrients that exist, for example.

The ability of nutrients and nutrition to mitigate the negative effects of space travel is far from being fully explored. Ground-based evidence is being amassed but is yet to be fully tested. In many ways, nutrition offers a suite of countermeasures that requires no more crew time than that already allotted for meals. While care clearly needs to be taken to avoid excessive amounts of any nutrient, the risks compared with those of pharmacological countermeasures are negligible.

This document details the nutrition evidence collected to date that has direct or indirect implications for space travelers. Although much has been done to obtain this evidence, a significant further effort will be required to validate the requirements listed herein, to optimize nutrition for astronauts, and ultimately to use this nutrition tool fully to help mitigate the health risks of microgravity exposure.

## **XVII. References**

1. Smith SM, Zwart SR. Nutritional biochemistry of spaceflight. *Adv Clin Chem.* 2008;46:87-130.
2. Smith SM, Zwart SR, Kloeris V, Heer M. Nutritional biochemistry of space flight. New York: Nova Science Publishers; 2009.
3. Bown SR. *Scurvy.* New York: St. Martin's Press; 2003.
4. National Aeronautics and Space Administration Johnson Space Center. Nutritional requirements for Extended Duration Orbiter missions (30-90 d) and Space Station Freedom (30-120 d). Report No.: JSC-32283. Houston, TX: National Aeronautics and Space Administration Lyndon B. Johnson Space Center 1993.
5. National Aeronautics and Space Administration Johnson Space Center. Nutritional requirements for International Space Station (ISS) missions up to 360 days. Report No.: JSC-28038. Houston, TX: National Aeronautics and Space Administration Lyndon B. Johnson Space Center 1996.
6. Smith SM, Zwart SR, Heer M. Human Adaptation to Spaceflight: The Role of Nutrition (NP-2014-10-018-JSC). Houston, TX: National Aeronautics and Space Administration Lyndon B. Johnson Space Center; 2014.
7. Bourland C, Kloeris V, Rice B, Vodovotz Y. Food systems for space and planetary flights. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models.* Boca Raton, FL: CRC Press; 2000. p. 19-40.
8. Perchonok M, Bourland C. NASA food systems: past, present, and future. *Nutrition.* 2002;18:913-20.
9. Heer M, Boerger A, Kamps N, Mika C, Korr C, Drummer C. Nutrient supply during recent European missions. *Pflugers Arch.* 2000;441:R8-14.
10. Lane HW, Kloeris V, Perchonok M, Zwart S, Smith SM. Food and nutrition for the moon base: what have we learned in 45 years of spaceflight. *Nutr Today.* 2007;42:102-10.
11. Klicka MV. Development of space foods. *J Am Diet Assoc.* 1964;44:358-61.
12. Klicka MV, Hollender HA, Lachance PA. Foods for astronauts. *J Am Diet Assoc.* 1967;51:238-45.
13. Smith MC, Berry CA. Dinner on the moon. *Nutr Today.* 1969;4:37-42.
14. LaChance PA, Berry CA. Luncheon in space. *Nutr Today* 1967:2-11.
15. Heidelbaugh ND. Space flight feeding concepts: characteristics, concepts for improvement, and public health implications. *J Am Vet Med Assoc.* 1966;149:1662-71.
16. Heidelbaugh ND, Vanderveen JE, Iger HG. Development and evaluation of a simplified formula food for aerospace feeding systems. *Aerosp Med.* 1968;39:38-43.
17. Gretebeck RJ, Siconolfi SF, Rice B, Lane HW. Physical performance is maintained in women consuming only foods used on the U.S. Space Shuttle. *Aviat Space Environ Med.* 1994;65:1036-40.
18. Institute of Medicine. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients).* Washington, DC: National Academy Press; 2002.
19. Waligora JM, Horrigan DJ. Metabolism and heat dissipation during Apollo EVA periods. In: Johnston RS, Dietlein LF, Berry CA, editors. *Biomedical results of Apollo (NASA SP-368).* Washington, DC: National Aeronautics and Space Administration; 1975. p. 115-28.
20. Lane HW, Gretebeck RJ, Smith SM. Nutrition, endocrinology, and body composition during space flight. *Nutr Res.* 1998;18:1923-34.
21. Lane HW, Gretebeck RJ, Schoeller DA, Davis-Street J, Socki RA, Gibson EK. Comparison of ground-based and space flight energy expenditure and water turnover in middle-aged healthy male US astronauts. *Am J Clin Nutr.* 1997;65:4-12.
22. Stein TP, Leskiw MJ, Schluter MD, Hoyt RW, Lane HW, Gretebeck RE, et al. Energy expenditure and balance during spaceflight on the space shuttle. *Am J Physiol Regul Integr Comp Physiol.* 1999;276:R1739-48.
23. Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E. Energy expenditure by doubly labeled water: validation in humans and proposed calculation. *Am J Physiol Regul Integr Comp Physiol.* 1986;250:R823-30.
24. Gretebeck RJ, Schoeller DA, Gibson EK, Lane HW. Energy expenditure during antiorthostatic bed rest (simulated microgravity). *J Appl Physiol.* 1995;78:2207-11.
25. Stein TP, Schluter MD, Leskiw MJ. Cortisol, insulin and leptin during space flight and bed rest. *J Gravit Physiol.* 1999;6:P85-6.
26. Lovejoy JC, Smith SR, Zachwieja JJ, Bray GA, Windhauser MM, Wickersham PJ, et al. Low-dose T(3) improves the bed rest model of simulated weightlessness in men and women. *Am J Physiol Endocrinol Metab.* 1999;277:E370-9.

## *Risk Factor of Inadequate Nutrition*

27. World Health Organization. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Geneva, Switzerland: World Health Organization; 1985.
28. Leach CS, Alfrey CP, Suki WN, Leonard JI, Rambaut PC, Inners LD, et al. Regulation of body fluid compartments during short-term spaceflight. *J Appl Physiol*. 1996;81:105-16.
29. Smith SM, Davis-Street JE, Rice BL, Nillen JL, Gillman PL, Block G. Nutritional status assessment in semiclosed environments: ground-based and space flight studies in humans. *J Nutr*. 2001;131:2053-61.
30. Smith SM, Wastney ME, O'Brien KO, Morukov BV, Larina IM, Abrams SA, et al. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the Mir space station. *J Bone Miner Res*. 2005;20:208-18.
31. Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE. The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. *J Nutr*. 2005;135:437-43.
32. Altman PL, Talbot JM. Nutrition and metabolism in spaceflight. *J Nutr*. 1987;117:421-7.
33. Johnson PC, Leach CS, Rambaut PC. Estimates of fluid and energy balances of Apollo 17. *Aerosp Med*. 1973;44:1227-30.
34. Rambaut PC, Smith MC, Jr, Wheeler HO. Nutritional studies. In: Johnston RS, Dietlein LF, Berry CA, editors. *Biomedical results of Apollo (NASA SP-368)*. Washington, DC: National Aeronautics and Space Administration; 1975. p. 277-302.
35. Rambaut PC, Leach CS, Johnson PC. Calcium and phosphorus change of the Apollo 17 crew members. *Nutr Metab*. 1975;18:62-9.
36. Stein TP, Schluter MD. Excretion of amino acids by humans during space flight. *Acta Astronaut*. 1998;42:205-14.
37. Heer M. Nutritional interventions related to bone turnover in European space missions and simulation models. *Nutrition*. 2002;18:853-6.
38. Rambaut P, Leach C, Leonard J. Observations in energy balance in man during spaceflight. *Am J Physiol*. 1977;233:R208-12.
39. Leach CS, Rambaut PC. Biochemical responses of the Skylab crewmen: an overview. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377)*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 204-16.
40. Drummer C, Hesse C, Baisch F, Norsk P, Elmann-Larsen B, Gerzer R, et al. Water and sodium balances and their relation to body mass changes in microgravity. *Eur J Clin Invest*. 2000;30:1066-75.
41. Smith SM, Heer MA, Shackelford L, Sibonga JD, Ploutz-Snyder L, Zwart SR. Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: evidence from biochemistry and densitometry. *J Bone Miner Res*. 2012;27:1896-906.
42. Smith SM, Lane HW. Spaceflight metabolism and nutritional support. In: Barratt MR, Pool SL, editors. *Principles of clinical medicine for space flight*. New York: Springer; 2008. p. 559-76.
43. Lane HW, Smith SM. Nutrition in space. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 783-8.
44. Agureev AN, Kalandarov S, Segal DE. [Optimization of cosmonauts' nutrition during the period of acute adaptation and at the closing stage of the mission]. *Aviakosm Ekolog Med*. 1997;31:47-51.
45. Olabi AA, Lawless HT, Hunter JB, Levitsky DA, Halpern BP. The effect of microgravity and space flight on the chemical senses. *J Food Sci*. 2002;67:468-78.
46. Baranski S, Kubiczkowa J, Piorko A, Skibniewski F, Bryanov, II, Milova EP, et al. Electrogustometric investigations during manned space flight. *Aviat Space Environ Med*. 1983;54:1-5.
47. Seddon MR, Fettman MJ, Phillips RW. Practical and clinical nutritional concerns during spaceflight. *Am J Clin Nutr*. 1994;60:825S-30S.
48. Watt DG, Money KE, Bondar RL, Thirsk RB, Garneau M, Scully-Power P. Canadian medical experiments on Shuttle flight 41-G. *Can Aeronaut Space J*. 1985;31:215-26.
49. Budyлина SM, Khvatova VA, Volozhin AI. Effect of orthostatic and antiorthostatic hypokinesia on taste sensitivity in men. *Kosm Biol Aviakosm Med*. 1976;10:27-30.
50. Kurliandskii V, Khvatova VA, Budyлина SM. [Functional mobility of taste receptors of the tongue under conditions of prolonged hypodynamia]. *Stomatologiia (Mosk)*. 1974;53:13-5.
51. Rice BL, Vickers ZM, Rose MS, Lane HW, editors. Fluid shifts during head-down bed rest do not influence flavor sensitivity. 67th Annual Scientific Meeting of the Aerospace Medical Association; 1996 May 5-9; Atlanta, GA.

## *Risk Factor of Inadequate Nutrition*

52. Lane HW, LeBlanc AD, Putcha L, Whitson PA. Nutrition and human physiological adaptations to space flight. *Am J Clin Nutr.* 1993;58:583-8.
53. Smirnov KV, Ugolev AM. Digestion and absorption. In: Leach Huntoon CS, Antipov VV, Grigoriev AI, editors. *Space biology and medicine. Volume III, Book 1, Humans in spaceflight.* Reston, VA: American Institute for Aeronautics and Astronautics; 1996. p. 211-30.
54. Heer M, Paloski WH. Space motion sickness: incidence, etiology, and countermeasures. *Auton Neurosci.* 2006;129:77-9.
55. Reschke MF, Bloomberg JJ, Harm DL, Paloski WH. Space flight and neurovestibular adaptation. *J Clin Pharmacol.* 1994;34:609-17.
56. Lackner JR, Dizio P. Space motion sickness. *Exp Brain Res.* 2006;175:377-99.
57. Nicogossian A. Medicine and space exploration. *Lancet.* 2003;362 Suppl:s8-9.
58. Smith SM, Zwart SR, Heer M, Hudson EK, Shackelford L, Morgan JL. Men and women in space: bone loss and kidney stone risk after long-duration spaceflight. *J Bone Miner Res.* 2014;29:1639-45.
59. Leach CS. Medical results from STS 1-4: analysis of body fluids. *Aviat Space Environ Med.* 1983;54(12 Suppl):S50-4.
60. Zwart SR, Launius RD, Coen GK, Morgan JLL, Charles JB, Smith SM. Body mass changes during long-duration spaceflight. *Aviat Space Environ Med.* 2014;85:897-904.
61. Lane HW, Schulz LO. Nutritional questions relevant to space flight. *Annu Rev Nutr.* 1992;12:257-78.
62. Leonard JL, Leach CS, Rambaut PC. Quantitation of tissue loss during prolonged space flight. *Am J Clin Nutr.* 1983;38:667-79.
63. Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, et al. Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. *Am J Physiol.* 1999;277:R1-10.
64. Johnston RS, Dietlein LF, Berry CA, editors. *Biomedical results of Apollo (NASA SP-368).* Washington, DC: National Aeronautics and Space Administration; 1975.
65. Brozek J, Grande F, Taylor HL, Anderson JT, Buskirk ER, Keys A. Changes in body weight and body dimensions in men performing work on a low calorie carbohydrate diet. *J Appl Physiol.* 1957;10:412-20.
66. Faintuch J, Soriano FG, Ladeira JP, Janiszewski M, Velasco IT, Gamma-Rodrigues JJ. Changes in body fluid and energy compartments during prolonged hunger strike. *Rev Hosp Clin Fac Med Sao Paulo.* 2000;55:47-54.
67. Blanc S, Somody L, Gharib C. Are energy metabolism alterations involved in cardiovascular deconditioning after weightlessness? An hypothesis. *Pflugers Arch.* 2000;441:R39-47.
68. Johnson PC, Driscoll TB, Alexander WC, Lambertsen CJ. Body fluid volume changes during a 14-day continuous exposure to 5.2% O<sub>2</sub> in N<sub>2</sub> at pressure equivalent to 100 FSW (4 ata). *Aerosp Med.* 1973;44:860-3.
69. Stein TP, Leskiw MJ, Schluter MD, Donaldson MR, Larina I. Protein kinetics during and after long-duration spaceflight on MIR. *Am J Physiol Endocrinol Metab.* 1999;276:E1014-21.
70. Biolo G, Ciocchi B, Stulle M, Bosutti A, Barazzoni R, Zanetti M, et al. Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest. *Am J Clin Nutr.* 2007;86:366-72.
71. Wade CE, Miller MM, Baer LA, Moran MM, Steele MK, Stein TP. Body mass, energy intake, and water consumption of rats and humans during space flight. *Nutrition.* 2002;18:829-36.
72. Florian J, Curren M, Baisch F, Pawelczyk J. Caloric restriction decreases orthostatic tolerance. *FASEB J.* 2004;18:478.6.
73. Ferrando AA, Paddon-Jones D, Wolfe RR. Alterations in protein metabolism during space flight and inactivity. *Nutrition.* 2002;18:837-41.
74. Oritsland NA. Starvation survival and body composition in mammals with particular reference to *Homo sapiens.* *Bull Math Biol.* 1990;52:643-55.
75. Korcok M. Hunger strikers may have died of fat, not protein, loss. *JAMA.* 1981;246:1878-9.
76. Leiter LA, Marliss EB. Survival during fasting may depend on fat as well as protein stores. *JAMA.* 1982;248:2306-7.
77. Phillips WJ. Starvation and survival: some military considerations. *Mil Med.* 1994;159:513-6.
78. Hughson RL, Shoemaker JK, Arbeille P, Dyson KS, Edgell H, Kerbeci P, et al. WISE 2005: vascular responses to 60-day bed rest in women. *J Gravit Physiol.* 2007;14:P53-4.
79. Lee SM, Schneider SM, Feiveson AH, Macias BR, Smith SM, Watenpaugh DE, et al. WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women. *J Appl Physiol.* 2014;116:654-67.

## *Risk Factor of Inadequate Nutrition*

80. Schneider SM, Lee SM, Macias BR, Watenpaugh DE, Hargens AR. WISE-2005: exercise and nutrition countermeasures for upright VO<sub>2</sub>pk during bed rest. *Med Sci Sports Exerc.* 2009;41:2165-76.
81. Smith SM, Zwart SR, Heer M, Lee SMC, Baecker N, Meuche S, et al. WISE-2005: Supine treadmill exercise within lower body negative pressure and flywheel resistive exercise as a countermeasure to bed rest-induced bone loss in women during 60-day simulated microgravity. *Bone.* 2008;42:572-81.
82. Francisco DR, Meck JV. Small Assessment Team (SAT) Report. Houston, TX: JSC2006.
83. Silber T. Anorexia nervosa: morbidity and mortality. *Pediatr Ann.* 1984;13:851, 5-9.
84. Garrow TS, Fletcher K, Halliday O. Body composition in severe infantile malnutrition. *J Clin Invest.* 1965;44:417-25.
85. Kerstetter JE, Caseria DM, Mitnick ME, Ellison AF, Gay LF, Liskov TA, et al. Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. *Am J Clin Nutr.* 1997;66:1188-96.
86. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein affects intestinal calcium absorption. *Am J Clin Nutr.* 1998;68:859-65.
87. Freudenheim JL, Johnson NE, Smith EL. Relationships between usual nutrient intake and bone-mineral content of women 35-65 years of age: longitudinal and cross-sectional analysis. *Am J Clin Nutr.* 1986;44:863-76.
88. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Protein consumption and bone mineral density in the elderly: the Rancho Bernardo Study. *Am J Epidemiol.* 2002;155:636-44.
89. Stein TP, Schluter MD. Plasma protein synthesis after spaceflight. *Aviat Space Environ Med.* 2006;77:745-8.
90. Rodriguez NR, DiMarco NM, Langley S. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. *J Am Diet Assoc.* 2009;109:509-27.
91. Cirillo M, De Santo NG, Heer M, Norsk P, Elmann-Larsen B, Bellini L, et al. Urinary albumin in space missions. *J Gravit Physiol.* 2002;9:P193-4.
92. Cirillo M, De Santo NG, Heer M, Norsk P, Elmann-Larsen B, Bellini L, et al. Low urinary albumin excretion in astronauts during space missions. *Nephron Physiol.* 2003;93:102-5.
93. Cirillo M, Stellato D, Heer M, Drummer C, Bellini L, De Santo NG. Urinary albumin in head-down bed rest. *J Gravit Physiol.* 2002;9:P195-6.
94. Whedon GD, Lutwak L, Rambaut PC, Whittle MW, Smith MC, Reid J, et al. Mineral and nitrogen metabolic studies, experiment M071. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377)*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 164-74.
95. Thornton WE, Rummel JA. Muscle deconditioning and its prevention in space flight. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377)*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 191-7.
96. Stein TP, Leskiw MJ, Schluter MD. Diet and nitrogen metabolism during spaceflight on the shuttle. *J Appl Physiol* (1985). 1996;81:82-97.
97. National Aeronautics and Space Administration Johnson Space Center. Nutrition requirements, standards, and operating bands for exploration missions. Report No.: JSC-63555. Houston, TX: Lyndon B. Johnson Space Center; 2005.
98. Zwart SR, Smith SM. The impact of space flight on the human skeletal system and potential nutritional countermeasures. *Intl SportMed J.* 2005;6:199-214.
99. Fettman MJ. Dietary instead of pharmacological management to counter the adverse effects of physiological adaptations to space flight. *Pflugers Arch.* 2000;441(2-3 Suppl):R15-20. Review.
100. Francescone R, Hou V, Grivennikov SI. Microbiome, inflammation, and cancer. *Cancer J.* 2014;20:181-9.
101. Frassetto L, Morris RC, Jr, Sellmeyer DE, Todd K, Sebastian A. Diet, evolution and aging--the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. *Eur J Nutr.* 2001;40:200-13.
102. Levine DS, Greenleaf JE. Immunosuppression during spaceflight deconditioning. *Aviat Space Environ Med.* 1998;69:172-7.
103. Stubbs RJ, Harbron CG, Murgatroyd PR, Prentice AM. Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. *Am J Clin Nutr.* 1995;62:316-29.
104. Jowsey J. Bone at the cellular level: the effects of inactivity (NASA SP-269). In: Murray RH, McCally M, editors. *Hypogravic and hypodynamic environments*. Washington, DC: National Aeronautics and Space Administration; 1971. p. 111-9.

105. Maaß H, Raabe W, Wegmann HM. Effects of microgravity on glucose tolerance. In: Sahm PR, Keller MH, Schiewe B, editors. Proceedings of the Norderney Symposium on Scientific Results of the German Spacelab Mission D-2. Norderney, Germany: Wissenschaftliche Projectführung D-2; 1995. p. 732-5.
106. Smirnov KV, Rubinova LG, Afonin BV, Noskov VB, Kravchenko VV. [Functional carbohydrate test during 237-day space flight]. *Kosm Biol Aviakosm Med.* 1991;25:61-2.
107. Alexandrov A, Gharib C, Grigoriev AI, Güell A, Kojarinov Y, Ruvina L, et al. [Oral glucose tolerance tests in man during a space flight of 150 days (Salyut 7-Soyuz T9)]. *C R Seances Soc Biol Fil.* 1985;179:192-5.
108. Biolo G, Ciochi B, Stulle M, Piccoli A, Lorenzon S, Dal Mas V, et al. Metabolic consequences of physical inactivity. *J Ren Nutr.* 2005;15:49-53.
109. Vernikos-Danellis J, Leach CS, Winget CM, Goodwin AL, Rambaut PC. Changes in glucose, insulin, and growth hormone levels associated with bedrest. *Aviat Space Environ Med.* 1976;47:583-7.
110. Stuart CA, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR. Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism.* 1988;37:802-6.
111. Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C. Fuel homeostasis during physical inactivity induced by bed rest. *J Clin Endocrinol Metab.* 2000;85:2223-33.
112. Stein TP, Schuler MD, Boden G. Development of insulin resistance by astronauts during spaceflight. *Aviat Space Environ Med.* 1994;65:1091-6.
113. Heer M, Baecker N, Wnendt S, Fischer A, Biolo G, Frings-Meuthen P. How fast is recovery of impaired glucose tolerance after 21-day bed rest (NUC Study) in healthy adults? *Sci World J.* 2014;803083; doi:10.1155/2014/
114. Lane HW, Rambaut PC. Nutrition. In: Nicogossian AE, Huntoon CL, Pool SL, editors. Space physiology and medicine. 3rd ed. Philadelphia: Lea & Febiger; 1994. p. 305-16.
115. Zerwekh JE, Odvina CV, Wuermser LA, Pak CY. Reduction of renal stone risk by potassium-magnesium citrate during 5 weeks of bed rest. *J Urol.* 2007;177:2179-84.
116. Pietrzyk RA, Jones JA, Sams CF, Whitson PA. Renal stone formation among astronauts. *Aviat Space Environ Med.* 2007;78:A9-13.
117. Pietrzyk RA, Feiveson AH, Whitson PA. Mathematical model to estimate risk of calcium-containing renal stones. *Miner Electrolyte Metab.* 1999;25:199-203.
118. Dolkas CB, Greenleaf JE. Insulin and glucose responses during bed rest with isotonic and isometric exercise. *J Appl Physiol.* 1977;43:1033-8.
119. Lipman RL, Ulvedal F, Schnure JJ, Bradley EM, Lecocq FR. Gluco-regulatory hormone response to 2-deoxy-d-glucose infusion in normal subjects at bedrest. *Metabolism.* 1970;19:980-7.
120. Sanders LM, Lupton JR. Carbohydrates. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 8th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 83-96.
121. Macdonald I, Williams CA. Effects of ingesting glucose and some of its polymers on serum glucose and insulin levels in men and women. *Ann Nutr Metab.* 1988;32:23-9.
122. Jacobs DR, Jr., Gallaher DD. Whole grain intake and cardiovascular disease: a review. *Curr Atheroscler Rep.* 2004;6:415-23.
123. Turner ND, Braby LA, Ford J, Lupton JR. Opportunities for nutritional amelioration of radiation-induced cellular damage. *Nutrition.* 2002;18:904-12.
124. Davidson LA, Nguyen DV, Hokanson RM, Callaway ES, Isett RB, Turner ND, et al. Chemopreventive n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Res.* 2004;64:6797-804.
125. Paulsrud JR, Pensler L, Whitten CF, Stewart S, Holman RT. Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. *Am J Clin Nutr.* 1972;25:897-904.
126. Holman RT. Polyunsaturated fatty acid profiles in human disease. In: Bazan NG, Paoletti R, Iacono JM, editors. New trends in nutrition, lipid research and cardiovascular diseases. New York: Alan R. Liss; 1981. p. 25-42.
127. Ritz P, Acheson KJ, Gachon P, Vico L, Bernard JJ, Alexandre C, et al. Energy and substrate metabolism during a 42-day bed-rest in a head-down tilt position in humans. *Eur J Appl Physiol Occup Physiol.* 1998;78:308-14.
128. Mazzucco S, Agostini F, Biolo G. Inactivity-mediated insulin resistance is associated with upregulated pro-inflammatory fatty acids in human cell membranes. *Clin Nutr.* 2010;29:386-90.

129. Macho L, Koska J, Ksinantova L, Pacak K, Hoff T, Noskov VB, et al. The response of endocrine system to stress loads during space flight in human subject. *Adv Space Res.* 2003;31:1605-10.
130. Stein TP, Schluter MD, Moldawer LL. Endocrine relationships during human spaceflight. *Am J Physiol Endocrinol Metab.* 1999;276:E155-62.
131. Chapkin RS, Davidson LA, Ly L, Weeks BR, Lupton JR, McMurray DN. Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. *J Nutr.* 2007;137:200S-4S.
132. Hong MY, Bancroft LK, Turner ND, Davidson LA, Murphy ME, Carroll RJ, et al. Fish oil decreases oxidative DNA damage by enhancing apoptosis in rat colon. *Nutr Cancer.* 2005;52:166-75.
133. Sanders LM, Henderson CE, Hong MY, Barhouni R, Burghardt RC, Wang N, et al. An increase in reactive oxygen species by dietary fish oil coupled with the attenuation of antioxidant defenses by dietary pectin enhances rat colonocyte apoptosis. *J Nutr.* 2004;134:3233-8.
134. Tisdale MJ. Molecular pathways leading to cancer cachexia. *Physiology (Bethesda).* 2005;20:340-8.
135. Whitehouse AS, Tisdale MJ. Downregulation of ubiquitin-dependent proteolysis by eicosapentaenoic acid in acute starvation. *Biochem Biophys Res Commun.* 2001;285:598-602.
136. Whitehouse AS, Smith HJ, Drake JL, Tisdale MJ. Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res.* 2001;61:3604-9.
137. Tisdale MJ. Loss of skeletal muscle in cancer: biochemical mechanisms. *Front Biosci.* 2001;6:D164-74.
138. Wigmore SJ, Barber MD, Ross JA, Tisdale MJ, Fearon KC. Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer.* 2000;36:177-84.
139. Smith HJ, Lorite MJ, Tisdale MJ. Effect of a cancer cachectic factor on protein synthesis/degradation in murine C2C12 myoblasts: modulation by eicosapentaenoic acid. *Cancer Res.* 1999;59:5507-13.
140. Wigmore SJ, Ross JA, Falconer JS, Plester CE, Tisdale MJ, Carter DC, et al. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition.* 1996;12(1 Suppl):S27-30.
141. Bayram I, Erbey F, Celik N, Nelson JL, Tanyeli A. The use of a protein and energy dense eicosapentaenoic acid containing supplement for malignancy-related weight loss in children. *Pediatr Blood Cancer.* 2009;52:571-4.
142. Tisdale MJ. Mechanisms of cancer cachexia. *Physiol Rev.* 2009;89:381-410.
143. Tisdale MJ. Cancer anorexia and cachexia. *Nutrition.* 2001;17:438-42.
144. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J.* 2007;6:2-10.
145. Orchard TS, Ing SW, Lu B, Belury MA, Johnson K, Wactawski-Wende J, et al. The association of red blood cell n-3 and n-6 fatty acids with bone mineral density and hip fracture risk in the women's health initiative. *J Bone Miner Res.* 2013;28:505-15.
146. Mangano K, Kerstetter J, Kenny A, Insogna K, Walsh SJ. An investigation of the association between omega 3 FA and bone mineral density among older adults: results from the National Health and Nutrition Examination Survey years 2005-2008. *Osteoporos Int.* 2014;25:1033-41.
147. Zwart SR, Pierson D, Mehta S, Gonda S, Smith SM. Capacity of omega-3 fatty acids or eicosapentaenoic acid to counteract weightlessness-induced bone loss by inhibiting NF-kappaB activation: from cells to bed rest to astronauts. *J Bone Miner Res.* 2010;25:1049-57.
148. Orchard TS, Cauley JA, Frank GC, Neuhauser ML, Robinson JG, Snetselaar L, et al. Fatty acid consumption and risk of fracture in the Women's Health Initiative. *Am J Clin Nutr.* 2010;92:1452-60.
149. Terano T. Effect of omega 3 polyunsaturated fatty acid ingestion on bone metabolism and osteoporosis. *World Rev Nutr Diet.* 2001;88:141-7.
150. Rousseau JH, Kleppinger A, Kenny AM. Self-reported dietary intake of omega-3 fatty acids and association with bone and lower extremity function. *J Am Geriatric Soc.* 2009;57:1781-8.
151. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *JAMA.* 2012;308:1024-33.
152. Andreeva VA, Touvier M, Kesse-Guyot E, Julia C, Galan P, Hercberg S. B vitamin and/or omega-3 fatty acid supplementation and cancer: ancillary findings from the supplementation with folate, vitamins B6 and B12, and/or omega-3 fatty acids (SU.FOL.OM3) randomized trial. *Arch Intern Med.* 2012;172:540-7.
153. Basse EJ, Littlewood JJ, Rothwell MC, Pye DW. Lack of effect of supplementation with essential fatty acids on bone mineral density in healthy pre- and postmenopausal women: two randomized controlled trials of Efacal v. calcium alone. *Br J Nutr.* 2000;83:629-35.

### *Risk Factor of Inadequate Nutrition*

154. Oh MS, Uribarri J. Electrolytes, water, and acid-base balance. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 105-40.
155. Sawka MN, Coyle EF. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exerc Sport Sci Rev*. 1999;27:167-218.
156. Sullivan RJ, Jr. Accepting death without artificial nutrition or hydration. *J Gen Intern Med*. 1993;8:220-4.
157. Leach Huntoon CS, Grigoriev AI, Natchin YV. Fluid and electrolyte regulation in spaceflight. San Diego: Univelt, Inc.; 1998.
158. Smith SM, Krauhs JM, Leach CS. Regulation of body fluid volume and electrolyte concentrations in spaceflight. *Adv Space Biol Med*. 1997;6:123-65.
159. Leach CS. A review of the consequences of fluid and electrolyte shifts in weightlessness. *Acta Astronaut*. 1979;6:1123-35.
160. Leach CS. An overview of the endocrine and metabolic changes in manned space flight. *Acta Astronaut*. 1981;8:977-86.
161. Leach CS. Fluid control mechanisms in weightlessness. *Aviat Space Environ Med*. 1987;58(9 Section II):A74-9.
162. Leach CS, Johnson PC, Jr. Fluid and electrolyte control in simulated and actual spaceflight. *Physiologist*. 1985;28(6 Suppl):S-34-7.
163. Drummer C, Gerzer R, Baisch F, Heer M. Body fluid regulation in micro-gravity differs from that on Earth: an overview. *Pflugers Arch*. 2000;441:R66-72.
164. De Santo NG, Christensen NJ, Drummer C, Kramer HJ, Regnard J, Heer M, et al. Fluid balance and kidney function in space: introduction. *Am J Kidney Dis*. 2001;38:664-7.
165. Norsk P, Drummer C, Christensen NJ, Cirillo M, Heer M, Kramer HJ, et al. Revised hypothesis and future perspectives. *Am J Kidney Dis*. 2001;38:696-8.
166. Gerzer R, Heer M. Regulation of body fluid and salt homeostasis - from observations in space to new concepts on Earth. *Curr Pharm Biotechnol*. 2005;6:299-304.
167. Norsk P, Christensen NJ, Bie P, Gabrielsen A, Heer M, Drummer C. Unexpected renal responses in space. *Lancet*. 2000;356:1577-8.
168. Nicogossian AE, Sawin CF, Huntoon CL. Overall physiologic response to space flight. In: Nicogossian AE, Huntoon CL, Pool SL, editors. *Space physiology and medicine*. 3rd ed. Philadelphia, PA: Lea & Febiger; 1994. p. 213-27.
169. Johnson PC, Driscoll TB, LeBlanc AD. Blood volume changes. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377)*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 235-41.
170. Bungo MW, Johnson PC, Jr. Cardiovascular examinations and observations of deconditioning during the space shuttle orbital flight test program. *Aviat Space Environ Med*. 1983;54:1001-4.
171. Vernikos J, Convertino VA. Advantages and disadvantages of fludrocortisone or saline load in preventing post-spaceflight orthostatic hypotension. *Acta Astronaut*. 1994;33:259-66.
172. Hyatt KH, West DA. Reversal of bedrest-induced orthostatic intolerance by lower body negative pressure and saline. *Aviat Space Environ Med*. 1977;48:120-4.
173. Heer M, Frings-Meuthen P, Titze J, Boschmann M, Frisch S, Baecker N, et al. Increasing sodium intake from a previous low or high intake affects water, electrolyte and acid-base differently. *Br J Nutr*. 2009;101:1286-94.
174. Thornton WE, Ord J. Physiological mass measurements in Skylab. (NASA SP-377) In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 175-82.
175. Leach CS, Inners LD, Charles JB. Changes in total body water during spaceflight. *J Clin Pharmacol*. 1991;31:1001-6.
176. Drummer C, Heer M, Dressendörfer RA, Strasburger CJ, Gerzer R. Reduced natriuresis during weightlessness. *Clin Investig*. 1993;71:678-86.
177. Norsk P. Cardiovascular and fluid volume control in humans in space. *Curr Pharm Biotechnol*. 2005;6:325-30.
178. Balakhovskiy I, Natchin Y. [Metabolism under the extreme conditions of spaceflight and during its simulation]. Moscow: Nauka Press; 1973.
179. Gerzer R, Drummer C, Heer M. Antinatriuretic kidney response to weightlessness. *Acta Astronaut*. 1994;33:97-100.

## *Risk Factor of Inadequate Nutrition*

180. Gerzer R, Heer M, Drummer C. Body fluid metabolism at actual and simulated microgravity. *Med Sci Sports Exerc.* 1996;28(10 Suppl):S32-5.
181. Whitson PA, Pietrzyk RA, Pak CY. Renal stone risk assessment during Space Shuttle flights. *J Urol.* 1997;158:2305-10.
182. Whitson P, Pietrzyk R, Pak C, Cintron N. Alterations in renal stone risk factors after space flight. *J Urol.* 1993;150:803-7.
183. Whitson PA, Pietrzyk RA, Morukov BV, Sams CF. The risk of renal stone formation during and after long duration space flight. *Nephron.* 2001;89:264-70.
184. Harm DL, Jennings RT, Meck JV, Powell MR, Putcha L, Sams CP, et al. Invited review: gender issues related to spaceflight: a NASA perspective. *J Appl Physiol.* 2001;91:2374-83.
185. Greenleaf JE. Mechanisms for negative water balance during weightlessness: immersion or bed rest? *Physiologist.* 1985;28(6 Suppl):S38-9.
186. Vernikos J. Metabolic and endocrine changes. In: Sandler H, Vernikos J, editors. *Inactivity: physiological effects.* Orlando, FL: Academic Press, Inc.; 1986. p. 99-121.
187. Norsk P. Renal adjustments to microgravity. *Pflugers Arch.* 2000;441:R62-5.
188. Norsk P, Christensen NJ, Vorobiev D, Suzuki Y, Drummer C, Heer M. Effects of head-down bed rest & microgravity on renal fluid excretion. *J Gravit Physiol.* 1998;5:P81-4.
189. Regnard J, Heer M, Drummer C, Norsk P. Validity of microgravity simulation models on earth. *Am J Kidney Dis.* 2001;38:668-74.
190. Institute of Medicine. *Dietary reference intakes for water, potassium, sodium, chloride, and sulfate.* Washington, DC: The National Academy Press; 2004.
191. Smith SM, McCoy T, Gazda D, Morgan JL, Heer M, Zwart SR. Space flight calcium: implications for astronaut health, spacecraft operations, and Earth. *Nutrients.* 2012;4:2047-68.
192. Tietz NW, Pruden EL, Siggaard-Andersen O. Electrolytes. In: Burtis CA, Ashwood ER, editors. *Tietz textbook of clinical chemistry.* 2nd ed. Philadelphia, PA: WB Saunders Company; 1994. p. 1887-973.
193. Preuss HG, Cloutre DL. Sodium, chloride, and potassium. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 8th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 475-92.
194. Pitts RF. *Ionic composition of body fluids. The physiological basis of diuretic therapy.* Springfield, IL: Charles C Thomas Publisher; 1959.
195. Lynn MP, Fouad F, Cook SA, Napoli CA, Ferrario CM. Alterations in cardiac function and cardiopulmonary blood volume in chronic sodium depletion in dogs. *Clin Sci (Lond).* 1980;59 Suppl 6:393s-5s.
196. Benedict FG. *A study of prolonged fasting.* Washington, DC: Carnegie Institution of Washington; 1915.
197. Keys AB, Brozek J, Henschel A. *The biology of human starvation.* Minneapolis: University of Minnesota Press; 1950.
198. Gamble JL, Ross GS, Tisdall FF. The metabolism of fixed base during fasting. *J Biol Chem.* 1923;57:633-95.
199. Heer M, Zittermann A, Hoetzel D. Role of nutrition during long-term spaceflight. *Acta Astronaut.* 1995;35:297-311.
200. Lane HW, Leach C, Smith SM. Fluid and electrolyte homeostasis. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models.* Boca Raton, FL: CRC Press; 2000. p. 119-39.
201. Machnik A, Dahlmann A, Kopp C, Goss J, Wagner H, van Rooijen N, et al. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension.* 2010;55:755-61.
202. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med.* 2009;15:545-52.
203. Rakova N, Juttner K, Dahlmann A, Schroder A, Linz P, Kopp C, et al. Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance. *Cell Metab.* 2013;17:125-31.
204. Titze J, Dahlmann A, Lerchl K, Kopp C, Rakova N, Schroder A, et al. Spooky sodium balance. *Kidney Int.* 2014;85:759-67.
205. Titze J, Krause H, Hecht H, Dietsch P, Rittweger J, Lang R, et al. Reduced osmotically inactive Na storage capacity and hypertension in the Dahl model. *Am J Physiol Renal Physiol.* 2002;283:F134-41.
206. Titze J, Shakibaei M, Schafflhuber M, Schulze-Tanzil G, Porst M, Schwind KH, et al. Glycosaminoglycan polymerization may enable osmotically inactive Na<sup>+</sup> storage in the skin. *Am J Physiol Heart Circ Physiol.* 2004;287:H203-8.

207. Ivanova LN, Archibasova VK, Shterental I. [Sodium-depositing function of the skin in white rats]. *Fiziol Zh SSSR Im I M Sechenova*. 1978;64:358-63.
208. Leach-Huntoon CS, Schneider H, Cintron NM, Landry R. Combined blood investigations. In: Bungo MW, Bagian TM, Bowman MA, Levitan BM, editors. *Results of the life sciences DSOs conducted aboard the Space Shuttle 1981-1986*. Houston: Space Biomedical Research Institute, Johnson Space Center; 1987. p. 7-11.
209. Goulding A. Effects of dietary NaCl supplements on parathyroid function, bone turnover and bone composition in rats taking restricted amounts of calcium. *Miner Electrolyte Metab*. 1980;4:203-8.
210. Goulding A, Lim PE. Effects of varying dietary salt intake on the fasting urinary excretion of sodium, calcium and hydroxyproline in young women. *N Z Med J*. 1983;96:853-4.
211. Evans CE, Chughtai AY, Blumsohn A, Giles M, Eastell R. The effect of dietary sodium on calcium metabolism in premenopausal and postmenopausal women. *Eur J Clin Nutr*. 1997;51:394-9.
212. Frassetto LA, Morris RC, Jr, Sellmeyer DE, Sebastian A. Adverse effects of sodium chloride on bone in the aging human population resulting from habitual consumption of typical American diets. *J Nutr*. 2008;138:419S-22S.
213. Castenmiller JJM, Mensink RP, van der Heijden L, Kouwenhoven T, Hautvast JG, de Leeuw PW, et al. The effect of dietary sodium on urinary calcium and potassium excretion in normotensive men with different calcium intakes. *Am J Clin Nutr*. 1985;41:52-60.
214. Cohen AJ, Roe FJ. Review of risk factors for osteoporosis with particular reference to a possible aetiological role of dietary salt. *Food Chem Toxicol*. 2000;38:237-53.
215. Heer M, Baisch F, Drummer C, Gerzer R. Long-term elevations of dietary sodium produce parallel increases in the renal excretion of urodilatin and sodium. In: Sahm PR, Keller MH, Schiewe B, editors. *Proceedings of the Norderney Symposium on Scientific Results of the German Spacelab Mission D-2*. Norderney, Germany: Wissenschaftliche Projectführung D-2; 1995. p. 708-14.
216. Ho SC, Chen YM, Woo JL, Leung SS, Lam TH, Janus ED. Sodium is the leading dietary factor associated with urinary calcium excretion in Hong Kong Chinese adults. *Osteoporos Int*. 2001;12:723-31.
217. Nordin B, Need A, Morris H, Horowitz M, Cochran M. Sodium and osteoporosis. In: Lesourd B, Rapin C, Sachet P, editors. *Osteoporose: pour une prevention nutritionnelle du risque?* Paris: Centre Recherche et Information Nutritionnelles; 1992. p. 117.
218. Nordin BE, Need AG, Morris HA, Horowitz M. The nature and significance of the relationship between urinary sodium and urinary calcium in women. *J Nutr*. 1993;123:1615-22.
219. Kleeman CR, Bohannon J, Bernstein D, Long S, Maxwell MH. Effect of variations in sodium intake on calcium excretion in normal humans. *Proc Soc Exp Biol Med*. 1964;115:29-32.
220. Heer M. Einfluss alimentärer erhöhter Kochsalzzufuhr auf den Wasser- und Elektrolythaushalt des Menschen [dissertation]. Bonn, Germany: University of Bonn; 1996.
221. Agus ZS, Goldfarb S. Renal regulation of calcium balance. In: Seldin DW, Giebisch G, editors. *The kidney: physiology and pathophysiology*. New York, NY: Raven Press; 1985. p. 1323-35.
222. Constanzo LS, Windhager EE. Effects of PTH, ADH and cyclic AMP on distal tubular Ca and Na reabsorption. *Am J Physiol*. 1980;239:F478-F85.
223. Meyer WJ, 3rd, Transbol I, Bartter FC, Delea C. Control of calcium absorption: effect of sodium chloride loading and depletion. *Metabolism*. 1976;9:989-93.
224. Breslau MA, McGuire J, Zerwekh J, Pak CYC. The role of dietary sodium on renal excretion and intestinal absorption of calcium and on vitamin D metabolism. *J Clin Endocrinol Metab*. 1982;55:369-73.
225. Ginty F, Flynn A, Cashman KD. The effect of dietary sodium intake on biochemical markers of bone metabolism in young women. *Br J Nutr*. 1998;79:343-50.
226. Lietz G, Avenell A, Robins SP. Short-term effects of dietary sodium intake on bone metabolism in postmenopausal women measured using urinary deoxypyridinoline excretion. *Br J Nutr*. 1997;78:73-82.
227. Blackwood AM, Sagnella GA, Cook DG, Cappuccio FP. Urinary calcium excretion, sodium intake and blood pressure in a multi-ethnic population: results of the Wandsworth Heart and Stroke Study. *J Hum Hypertens*. 2001;15:229-37.
228. Harrington M, Cashman KD. High salt intake appears to increase bone resorption in postmenopausal women but high potassium intake ameliorates this adverse effect. *Nutr Rev*. 2003;61:179-83.
229. Harrington M, Bennett T, Jakobsen J, Ovesen L, Brot C, Flynn A, et al. The effect of a high-protein, high-sodium diet on calcium and bone metabolism in postmenopausal women and its interaction with vitamin D receptor genotype. *Br J Nutr*. 2004;91:41-51.

230. Harrington M, Bennett T, Jakobsen J, Ovesen L, Brot C, Flynn A, et al. Effect of a high-protein, high-salt diet on calcium and bone metabolism in postmenopausal women stratified by hormone replacement therapy use. *Eur J Clin Nutr.* 2004;58:1436-9.
231. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and stone risk. *Nutr Rev.* 1995;53:131-9.
232. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and bone loss. *J Bone Miner Res.* 1996;11:731-6.
233. Sellmeyer DE, Schloetter M, Sebastian A. Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. *J Clin Endocrinol Metab.* 2002;87:2008-12.
234. Nordin BE, Need AG, Steurer T, Morris HA, Chatterton BE, Horowitz M. Nutrition, osteoporosis, and aging. *Ann N Y Acad Sci.* 1998;854:336-51.
235. de Wardener HE, MacGregor GA. Harmful effects of dietary salt in addition to hypertension. *J Hum Hypertens.* 2002;16:213-23.
236. Fellstrom B, Danielson BG, Karlstrom B, Lithell H, Ljunghal S, Vessby B. Dietary habits in renal stone patients compared with healthy subjects. *Br J Urol.* 1989;63:575-80.
237. Trinchieri A, Mandressi A, Luongo P, Longo G, Pisani E. The influence of diet on urinary risk factors for stones in healthy subjects and idiopathic renal calcium stone formers. *Br J Urol.* 1991;67:230-6.
238. Sakhaee K, Harvey JA, Padalino PK, Whitson P, Pak CY. The potential role of salt abuse on the risk for kidney stone formation. *J Urol.* 1993;150(2 Pt 1):310-2.
239. Matkovic V, Ilich JZ, Andon MB, Hsieh LC, Tzagournis MA, Lagger BJ, et al. Urinary calcium, sodium, and bone mass of young females. *Am J Clin Nutr.* 1995;62:417.
240. Devine A, Criddle RA, Dick IM, Kerr DA, Prince RL. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. *Am J Clin Nutr.* 1995;62:740-5.
241. Frings-Meuthen P, Baecker N, Heer M. Low-grade metabolic acidosis may be the cause of sodium chloride-induced exaggerated bone resorption. *J Bone Miner Res.* 2008;23:517-24.
242. Frings-Meuthen P, Buehlmeier J, Baecker N, Stehle P, Fimmers R, May F, et al. High sodium chloride intake exacerbates immobilization-induced bone resorption and protein losses. *J Appl Physiol.* 2011;111:537-42.
243. Arnaud SB, Wolinsky I, Fung P, Vernikos J. Dietary salt and urinary calcium excretion in a human bed rest spaceflight model. *Aviat Space Environ Med.* 2000;71:1115-9.
244. May RC, Kelly RA, Mitch WE. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. *J Clin Invest.* 1986;77:614-21.
245. Buehlmeier J, Frings-Meuthen P, Remer T, Maser-Gluth C, Stehle P, Biolo G, et al. Alkaline salts to counteract bone resorption and protein wasting induced by high salt intake: results of a randomized controlled trial. *J Clin Endocrinol Metab.* 2012;97:4789-97.
246. Compston J. Management of glucocorticoid-induced osteoporosis. *Nat Rev Rheumatol.* 2010;6:82-8.
247. Cooper MS, Walker EA, Bland R, Fraser WD, Hewison M, Stewart PM. Expression and functional consequences of 11beta-hydroxysteroid dehydrogenase activity in human bone. *Bone.* 2000;27:375-81.
248. Dovio A, Perazzolo L, Osella G, Ventura M, Termine A, Milano E, et al. Immediate fall of bone formation and transient increase of bone resorption in the course of high-dose, short-term glucocorticoid therapy in young patients with multiple sclerosis. *J Clin Endocrinol Metab.* 2004;89:4923-8.
249. Kuroki Y, Kaji H, Kawano S, Kanda F, Takai Y, Kajikawa M, et al. Short-term effects of glucocorticoid therapy on biochemical markers of bone metabolism in Japanese patients: a prospective study. *J Bone Miner Metab.* 2008;26:271-8.
250. Vormann J, Remer T. Dietary, metabolic, physiologic, and disease-related aspects of acid-base balance: foreword to the contributions of the second International Acid-Base Symposium. *J Nutr.* 2008;138:413S-4S.
251. Burckhardt P. The effect of the alkali load of mineral water on bone metabolism: interventional studies. *J Nutr.* 2008;138:435S-7S.
252. Arnett TR. Extracellular pH regulates bone cell function. *J Nutr.* 2008;138:415S-8S.
253. Kleinman LI, Lorenz JM. Physiology and pathophysiology of body water and electrolytes. In: Kaplan LA, Pesce AJ, editors. *Clinical chemistry: theory, analysis, and correlation.* St. Louis, MO: CV Mosby Company; 1984. p. 363-86.
254. Perez G, Delaney VB, Bourke E. Hypo- and hyperkalemia. In: Preuss HG, editor. *Management of common problems in renal disease.* Philadelphia, PA: Field and Wood Inc; 1988. p. 109-17.
255. Srivastava TN, Young DB. Impairment of cardiac function by moderate potassium depletion. *J Card Fail.* 1995;1:195-200.

## *Risk Factor of Inadequate Nutrition*

256. Whatham A, Bartlett H, Eperjesi F, Blumenthal C, Allen J, Suttle C, et al. Vitamin and mineral deficiencies in the developed world and their effect on the eye and vision. *Ophthalmic Physiol Opt.* 2008;28:1-12.
257. van Poppel G, Goldbohm RA. Epidemiologic evidence for beta-carotene and cancer prevention. *Am J Clin Nutr.* 1995;62 Suppl:1393S-402S.
258. Kohlmeier L, Hastings SB. Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. *Am J Clin Nutr.* 1995;62 Suppl:1370S-6S.
259. Olson JA. Vitamin A, retinoids, and carotenoids. In: Shils ME, Olson JA, Shike M, editors. *Modern nutrition in health and disease.* 8th ed. Malvern, PA: Lea & Febiger; 1994. p. 287-307.
260. Solomons NW, Russell RM. "Appropriate technology" for vitamin A field research. *Am J Clin Nutr.* 2001;73:849-50.
261. Ross AC. Vitamin A and retinoids. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease.* 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 305-27.
262. Leo MA, Lieber CS. Alcohol, vitamin A, and beta-carotene: adverse interactions, including hepatotoxicity and carcinogenicity. *Am J Clin Nutr.* 1999;69:1071-85.
263. Leo MA, Lieber CS. Hepatic vitamin A depletion in alcoholic liver injury. *N Engl J Med.* 1982;307:597-601.
264. Michaelsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med.* 2003;348:287-94.
265. Melhus H, Michaelsson K, Kindmark A, Bergstrom R, Holmberg L, Mallmin H, et al. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med.* 1998;129:770-8.
266. Jackson HA, Sheehan AH. Effect of vitamin A on fracture risk. *Ann Pharmacother.* 2005;39:2086-90.
267. Palacios C. The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutr.* 2006;46:621-8.
268. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst.* 1996;88:1550-9.
269. ATBC Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. *Ann Epidemiol.* 1994;4:1-10.
270. Rastegar N, Eckart P, Mertz M. Radiation-induced cataract in astronauts and cosmonauts. *Graefes Arch Clin Exp Ophthalmol.* 2002;240:543-7.
271. Cucinotta FA, Manuel FK, Jones J, Iszard G, Murrey J, Djojonegoro B, et al. Space radiation and cataracts in astronauts. *Radiat Res.* 2001;156:460-6.
272. Chylack LT, Jr., Peterson LE, Feiveson AH, Wear ML, Manuel FK, Tung WH, et al. NASA study of cataract in astronauts (NASCA). Report 1: cross-sectional study of the relationship of exposure to space radiation and risk of lens opacity. *Radiat Res.* 2009;172:10-20.
273. Jones JA, McCarten M, Manuel K, Djojonegoro B, Murray J, Feiversen A, et al. Cataract formation mechanisms and risk in aviation and space crews. *Aviat Space Environ Med.* 2007;78:A56-66.
274. Chylack LT, Jr., Feiveson AH, Peterson LE, Tung WH, Wear ML, Marak LJ, et al. NASCA Report 2: longitudinal study of relationship of exposure to space radiation and risk of lens opacity. *Radiat Res.* 2012;178:25-32.
275. Agte V, Tarwadi K. The importance of nutrition in the prevention of ocular disease with special reference to cataract. *Ophthalmic Res.* 2010;44:166-72.
276. Cui YH, Jing CX, Pan HW. Association of blood antioxidants and vitamins with risk of age-related cataract: a meta-analysis of observational studies. *Am J Clin Nutr.* 2013;98:778-86.
277. Institute of Medicine. *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.* Washington, DC: National Academy Press; 2001.
278. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol.* 2005;289:F8-28.
279. Institute of Medicine. *Dietary reference intakes for calcium and vitamin D.* Washington, DC: National Academy Press; 2011.
280. Aloia JF. The 2011 Report on Dietary Reference Intake for Vitamin D: Where do we go from here? *J Clin Endocrinol Metab.* 2011;96:2987-96.
281. Davis CD, Hartmuller V, Freedman DM, Hartge P, Picciano MF, Swanson CA, et al. Vitamin D and cancer: current dilemmas and future needs. *Nutr Rev.* 2007;65:S71-4.
282. Holick MF. Vitamin D deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. *Nat Rev Endocrinol.* 2011;7:73-5.

## *Risk Factor of Inadequate Nutrition*

283. Bikle DD. Vitamin D regulation of immune function. *Vitam Horm*. 2011;86:1-21.
284. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab*. 2009;94:26-34.
285. Davis CD, Milner JA. Vitamin D and colon cancer. *Expert Rev Gastroenterol Hepatol*. 2011;5:67-81.
286. Scragg R. Vitamin D and public health: an overview of recent research on common diseases and mortality in adulthood. *Public Health Nutr*. 2011;14:1515-32.
287. Grant WB. An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. *Cancer*. 2002;94:272-81.
288. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control*. 2000;11:847-52.
289. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer*. 2002;94:1867-75.
290. Hewison M, Zehnder D, Chakraverty R, Adams JS. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. *Mol Cell Endocrinol*. 2004;215:31-8.
291. Norman AW, Henry HL. Vitamin D. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition*. 8th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 199-213.
292. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res*. 2007;22 Suppl 2:V28-33.
293. Gilchrest BA. Sun exposure and vitamin D sufficiency. *Am J Clin Nutr*. 2008;88:570S-7S.
294. Mohr SB, Garland CF, Gorham ED, Grant WB, Garland FC. Relationship between low ultraviolet B irradiance and higher breast cancer risk in 107 countries. *Breast J*. 2008;14:255-60.
295. Gilchrest BA. Sun protection and Vitamin D: three dimensions of obfuscation. *J Steroid Biochem Mol Biol*. 2007;103:655-63.
296. Smith SM, Davis-Street JE, Fesperman JV, Calkins DS, Bawa M, Macias BR, et al. Evaluation of treadmill exercise in a lower body negative pressure chamber as a countermeasure for weightlessness-induced bone loss: a bed rest study with identical twins. *J Bone Miner Res*. 2003;18:2223-30.
297. Muller HK, Lugg DJ, Quinn D. Cell mediated immunity in Antarctic wintering personnel; 1984-1992. *Immunol Cell Biol*. 1995;73:316-20.
298. Muller HK, Lugg DJ, Ursin H, Quinn D, Donovan K. Immune responses during an Antarctic summer. *Pathology (Phila)*. 1995;27:186-90.
299. Shirai T, Magara KK, Motohashi S, Yamashita M, Kimura M, Suwazomo Y, et al. TH1-biased immunity induced by exposure to Antarctic winter. *J Allergy Clin Immunol*. 2003;111:1353-60.
300. Oliveri MB, Mautalen C, Bustamante L, Gomez Garcia V. Serum levels of 25-hydroxyvitamin D in a year of residence on the Antarctic continent. *Eur J Clin Nutr*. 1994;48:397-401.
301. Yonei T, Hagino H, Katagiri H, Kishimoto H. Bone metabolic changes in Antarctic wintering team members. *Bone*. 1999;24:145-50.
302. Lisbona Gil A, Fernandez Riestra FA, Contreras Fernandez R, Herrero Huertas E, Martinez Gomez ME. [Concentrations of 25-hydroxyvitamin D3 in Antarctica]. *Med Clin (Barc)*. 1992;99:206-9.
303. Zerath E, Holy X, Gaud R, Schmitt D. Decreased serum levels of 1,25-(OH)<sub>2</sub> vitamin D during 1 year of sunlight deprivation in the Antarctic. *Eur J Appl Physiol Occup Physiol*. 1999;79:141-7.
304. Pitson GA, Lugg DJ, Roy CR. Effect of seasonal ultraviolet radiation fluctuations on vitamin D homeostasis during an Antarctic expedition. *Eur J Appl Physiol Occup Physiol*. 1996;72:231-4.
305. Rettberg P, Horneck G, Zittermann A, Heer M. Biological dosimetry to determine the UV radiation climate inside the MIR station and its role in vitamin D biosynthesis. *Adv Space Res*. 1998;22:1643-52.
306. Reed HL, Reedy KR, Palinkas LA, Van Do N, Finney NS, Case HS, et al. Impairment in cognitive and exercise performance during prolonged antarctic residence: effect of thyroxine supplementation in the polar triiodothyronine syndrome. *J Clin Endocrinol Metab*. 2001;86:110-6.
307. Farrace S, Cenni P, Tuoizzi G, Casagrande M, Barbarito B, Peri A. Endocrine and psychophysiological aspects of human adaptation to the extreme. *Physiol Behav*. 1999;66:613-20.
308. Gloth FM, 3rd, Alam W, Hollis B. Vitamin D vs broad spectrum phototherapy in the treatment of seasonal affective disorder. *J Nutr Health Aging*. 1999;3:5-7.
309. Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *J Intern Med*. 2000;247:260-8.
310. Duplessis CA, Harris EB, Watenpaugh DE, Horn WG. Vitamin D supplementation in underway submariners. *Aviat Space Environ Med*. 2005;76:569-75.

## *Risk Factor of Inadequate Nutrition*

311. Smith SM, Gardner KK, Locke J, Zwart SR. Vitamin D supplementation during Antarctic winter. *Am J Clin Nutr.* 2009;89:1092-8.
312. Zwart SR, Mehta SK, Ploutz-Snyder R, Bourbeau Y, Locke JP, Pierson DL, et al. Response to vitamin D supplementation during Antarctic winter is related to BMI, and supplementation can mitigate Epstein-Barr virus reactivation. *J Nutr.* 2011;141:692-7.
313. Zwart SR, Parsons H, Kimlin M, Innis SM, Locke JP, Smith SM. A 250 µg/week dose of vitamin D was as effective as a 50 µg/d dose in healthy adults, but a regimen of four weekly followed by monthly doses of 1250 µg raised the risk of hypercalciuria. *Br J Nutr.* 2013;110:1866-72.
314. Smith SM, Heer M, Zwart SR. Nutrition and bone health in space. In: Holick M, Nieves J, editors. *Nutrition and bone health*, 2nd ed. New York: Springer; in press.
315. Bjorkman M, Sorva A, Risteli J, Tilvis R. Vitamin D supplementation has minor effects on parathyroid hormone and bone turnover markers in vitamin D-deficient bedridden older patients. *Age Ageing.* 2008;37:25-31.
316. Sorva A, Valimaki M, Risteli J, Risteli L, Elfving S, Takkunen H, et al. Serum ionized calcium, intact PTH and novel markers of bone turnover in bedridden elderly patients. *Eur J Clin Invest.* 1994;24:806-12.
317. Thys-Jacobs S, Chan FKW, Koberle LMC, et al. Hypercalcemia due to vitamin D toxicity. In: Feldman D, Glorieux FH, Pike JW, editors. *Vitamin D*. San Diego, CA: Academic Press; 1997. p. 883-901.
318. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr.* 2007;85:6-18.
319. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr.* 1999;69:842-56.
320. Vieth R. Vitamin D toxicity, policy, and science. *J Bone Miner Res.* 2007;22 Suppl 2:V64-8.
321. Traber MG. Vitamin E. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition*. 7th ed. Washington, DC: International Life Sciences Institute; 2010. p. 214-29.
322. Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academy Press; 2000.
323. Kayden HJ, Traber MG. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res.* 1993;34:343-58.
324. Traber MG. Vitamin E inadequacy in humans: causes and consequences. *Adv Nutr.* 2014;5:503-14.
325. Smith BJ, Lucas EA, Turner RT, Evans GL, Lerner MR, Brackett DJ, et al. Vitamin E provides protection for bone in mature hindlimb unloaded male rats. *Calcif Tissue Int.* 2005;76:272-9.
326. Stein TP. Space flight and oxidative stress. *Nutrition.* 2002;18:867-71.
327. Stein TP, Leski MJ. Oxidant damage during and after spaceflight. *Am J Physiol Endocrinol Metab.* 2000;278:E375-82.
328. Fang Y, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition.* 2002;18:872-9.
329. Pence BC, Yang TC. Antioxidants: radiation and stress. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models*. Boca Raton, FL: CRC Press; 2000. p. 233-52.
330. Olson RE. The function and metabolism of vitamin K. *Annu Rev Nutr.* 1984;4:281-337.
331. Shearer MJ, Fu X, Booth SL. Vitamin K nutrition, metabolism, and requirements: current concepts and future research. *Adv Nutr.* 2012;3:182-95.
332. Gundberg CM, Lian JB, Booth SL. Vitamin K-dependent carboxylation of osteocalcin: friend or foe? *Adv Nutr.* 2012;3:149-57.
333. Ferland G. Vitamin K. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition*. 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 230-47.
334. Hodges SJ, Bejui J, Leclercq M, Delmas PD. Detection and measurement of vitamins K1 and K2 in human cortical and trabecular bone. *J Bone Miner Res.* 1993;8:1005-8.
335. Usui Y, Tanimura H, Nishimura N, Kobayashi N, Okanoue T, Ozawa K. Vitamin K concentrations in the plasma and liver of surgical patients. *Am J Clin Nutr.* 1990;51:846-52.
336. Majerus PW, Broze GJ, Miletech JP, Tollefsen DM. Anticoagulant, thrombolytic, and antiplatelet drugs. In: Gilman AG, Rall TW, Nies AS, Taylor P, editors. *The pharmacological basis of therapeutics*. 8th ed. New York: Pergamon Press; 1990. p. 1311-31.
337. Kempin SJ. Warfarin resistance caused by broccoli. *N Engl J Med.* 1983;308:1229-30.
338. Haywood LJ. Coronary heart disease mortality/morbidity and risk in blacks. I: Clinical manifestations and diagnostic criteria: the experience with the Beta Blocker Heart Attack Trial. *Am Heart J.* 1984;108:787-93.
339. Caillot-Augusseau A, Vico L, Heer M, Voroviev D, Souberbielle J-C, Zitterman A, et al. Space flight is associated with rapid decreases of undercarboxylated osteocalcin and increases of markers of bone

## *Risk Factor of Inadequate Nutrition*

- resorption without changes in their circadian variation: observations in two cosmonauts. *Clin Chem.* 2000;46:1136-43.
340. Vermeer C, Wolf J, Craciun AM, Knapen MH. Bone markers during a 6-month space flight: effects of vitamin K supplementation. *J Gravit Physiol.* 1998;5:65-9.
341. Zwart SR, Booth SL, Peterson JW, Wang Z, Smith SM. Vitamin K status in spaceflight and ground-based models of spaceflight. *J Bone Miner Res.* 2011;26:948-54.
342. Shearer MJ. Vitamin K. *Lancet.* 1995;345:229-34.
343. Caraballo PJ, Heit JA, Atkinson EJ, Silverstein MD, O'Fallon WM, Castro MR, et al. Long-term use of oral anticoagulants and the risk of fracture. *Arch Intern Med.* 1999;159:1750-6.
344. Bailey LB, Caudill MA. Folate. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 9th ed. Washington, DC: International Life Sciences Institute; 2010. p. 321-42.
345. Groff J, Gropper S. *Advanced nutrition and human metabolism.* 3rd ed. St. Paul, MN: Wadsworth Publishing; 2000.
346. Herbert V. Development of human folate deficiency. In: Picciano MF, Sotokstad ELR, Gregory JFI, editors. *Folic acid metabolism in health and disease.* New York: Wiley-Liss; 1990. p. 195-210.
347. Herbert V. Folic acid. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease.* 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999.
348. Institute of Medicine. *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline.* Washington, DC: National Academy Press; 2000.
349. Zwart SR, Gibson CR, Mader TH, Ericson K, Ploutz-Snyder R, Heer M, et al. Vision changes after spaceflight are related to alterations in folate- and vitamin B-12-dependent one-carbon metabolism. *J Nutr.* 2012;142:427-31.
350. Endoh K, Murakami M, Araki R, Maruyama C, Umegaki K. Low folate status increases chromosomal damage by X-ray irradiation. *Int J Radiat Biol.* 2006;82:223-30.
351. Endoh K, Murakami M, Umegaki K. Vulnerability of folate in plasma and bone marrow to total body irradiation in mice. *Int J Radiat Biol.* 2007;83:65-71.
352. Beetstra S, Thomas P, Salisbury C, Turner J, Fenech M. Folic acid deficiency increases chromosomal instability, chromosome 21 aneuploidy and sensitivity to radiation-induced micronuclei. *Mutat Res.* 2005;578:317-26.
353. Courtemanche C, Huang AC, Elson-Schwab I, Kerry N, Ng BY, Ames BN. Folate deficiency and ionizing radiation cause DNA breaks in primary human lymphocytes: a comparison. *FASEB J.* 2004;18:209-11.
354. Joshi R, Adhikari S, Patro BS, Chattopadhyay S, Mukherjee T. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radic Biol Med.* 2001;30:1390-9.
355. Zwart SR, Jessup JM, Ji J, Smith SM. Saturation diving alters folate status and biomarkers of DNA damage and repair. *PLoS One.* 2012;7:e31058.
356. Zwart SR, Crawford GE, Gillman PL, Kala G, Rodgers AS, Rogers A, et al. Effects of 21 days of bed rest, with or without artificial gravity, on nutritional status of humans. *J Appl Physiol.* 2009;107:54-62.
357. Zwart SR, Oliver SM, Feserman JV, Kala G, Krauhs J, Ericson K, et al. Nutritional status assessment before, during, and after long-duration head-down bed rest. *Aviat Space Environ Med.* 2009;80:A15-22.
358. Smith SM, Davis-Street JE, Feserman JV, Smith MD, Rice BL, Zwart SR. Nutritional assessment during a 14-d saturation dive: the NASA Extreme Environment Mission Operations V Project. *J Nutr.* 2004;134:1765-71.
359. Caballero B, Allen L, Prentice A. *Encyclopedia of human nutrition.* 2nd ed. Oxford, UK: Elsevier Academic Press; 2005.
360. Volpe SL, King JC, Coburn SP. Micronutrients: trace elements and B vitamins. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models.* Boca Raton, FL: CRC Press; 2000. p. 213-32.
361. Fox JB, Jr., Thayer DW, Jenkins RK, Phillips JG, Ackerman SA, Beecher GR, et al. Effect of gamma irradiation on the B vitamins of pork chops and chicken breasts. *Int J Radiat Biol.* 1989;55:689-703.
362. Bychkov VP, Kozar MI, Popov VI, Boiko NN, Kolchin EV. [Experimental data on the effect of proton and gamma radiation on food products]. *Kosm Biol Aviakosm Med.* 1974;8:6-10.
363. Burger IH, Walters CL. The effect of processing on the nutritive value of flesh foods. *Proc Nutr Soc.* 1973;32:1-8.
364. De Groot AP, Van der Mijll Dekker LP, Slump P, Vos HJ, Willems JLL. *Composition and nutritive value of radiation-pasteurized chicken.* The Netherlands: Central Institute for Nutrition and Food Research 1972 Contract No.: Report No. R3787.

365. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr.* 2003;77:1352-60.
366. Singer TP, Kenney WC. Biochemistry of covalently bound flavins. *Vitam Horm.* 1974;32:1-45.
367. Hughes SG, Riis RC, Nickum JG, Rumsey GL. Biomicroscopic and histologic pathology of the eye in riboflavin deficient rainbow trout (*Salmogairdneri*). *Cornell Vet.* 1981;71:269-79.
368. Bates CJ, Liu DS, Fuller NJ, Lucas A. Susceptibility of riboflavin and vitamin A in breast milk to photodegradation and its implications for the use of banked breast milk in infant feeding. *Acta Paediatr Scand.* 1985;74:40-4.
369. Lakritz L, Fox JB, Thayer DW. Thiamin, riboflavin, and alpha-tocopherol content of exotic meats and loss due to gamma radiation. *J Food Prot.* 1998;61:1681-3.
370. Henshall JD. The effect of processing on the nutritive value of fruit and vegetable products. *Proc Nutr Soc.* 1973;32:17-22.
371. Fan X, Thayer DW. gamma-Radiation influences browning, antioxidant activity, and malondialdehyde level of apple juice. *J Agric Food Chem.* 2002;50:710-5.
372. Salem SA. Effect of gamma radiation on the storage of onions used in the dehydration industry. *J Sci Food Agric.* 1974;25:257-62.
373. Calucci L, Pinzino C, Zandomenighi M, Capocchi A, Ghiringhelli S, Saviozzi F, et al. Effects of gamma-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J Agric Food Chem.* 2003;51:927-34.
374. Jagetia GC, Rajanikant GK, Baliga MS, Rao KV, Kumar P. Augmentation of wound healing by ascorbic acid treatment in mice exposed to gamma-radiation. *Int J Radiat Biol.* 2004;80:347-54.
375. Cai L, Koropatnick J, Cherian MG. Roles of vitamin C in radiation-induced DNA damage in presence and absence of copper. *Chem Biol Interact.* 2001;137:75-88.
376. Buettner GR, Jurkiewicz BA. Catalytic metals, ascorbate and free radicals: combinations to avoid. *Radiat Res.* 1996;145:532-41.
377. Da Silva VR, Russell KA, Gregory III JF. Vitamin B-6. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 8th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 307-20.
378. Shane B, editor. *Vitamin B6 and blood. Human vitamin B6 requirements: proceedings of a workshop;* 1978; Washington, DC: National Academy Press.
379. Kraemer WJ, Staron RS, Gordon SE, Volek JS, Koziris LP, Duncan ND, et al. The effects of 10 days of spaceflight on the shuttle Endeavor on predominantly fast-twitch muscles in the rat. *Histochem Cell Biol.* 2000;114:349-55.
380. Coburn SP, Lewis DL, Fink WJ, Mahuren JD, Schaltenbrand WE, Costill DL. Human vitamin B-6 pools estimated through muscle biopsies. *Am J Clin Nutr.* 1988;48:291-4.
381. Coburn SP, Thampy KG, Lane HW, Conn PS, Ziegler PJ, Costill DL, et al. Pyridoxic acid excretion during low vitamin B-6 intake, total fasting, and bed rest. *Am J Clin Nutr.* 1995;62:979-83.
382. Hvas AM, Juul S, Bech P, Nexø E. Vitamin B6 level is associated with symptoms of depression. *Psychother Psychosom.* 2004;73:340-3.
383. Bassler KH. Use and abuse of high dosages of vitamin B6. *Int J Vitam Nutr Res Suppl.* 1989;30:120-6.
384. Gdynia HJ, Muller T, Sperfeld AD, Kuhnlein P, Otto M, Kassubek J, et al. Severe sensorimotor neuropathy after intake of highest dosages of vitamin B6. *Neuromuscul Disord.* 2008;18:156-8.
385. Katan MB. [How much vitamin B6 is toxic?]. *Ned Tijdschr Geneesk.* 2005;149:2545-6.
386. Shin YS, Beuhring KU, Stokstad EL. The relationships between vitamin B12 and folic acid and the effect of methionine on folate metabolism. *Mol Cell Biochem.* 1975;9:97-108.
387. van Asselt DZ, van den Broek WJ, Lamers CB, Corstens FH, Hoefnagels WH. Free and protein-bound cobalamin absorption in healthy middle-aged and older subjects. *J Am Geriatr Soc.* 1996;44:949-53.
388. Stabler SP. Vitamin B<sub>12</sub>. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 343-57.
389. Chu C, Scanlon P. Vitamin B12 deficiency optic neuropathy detected by asymptomatic screening. *BMJ Case Rep [serial on the Internet].* 2011 3083013; 2011: Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22696671>.
390. Jalil A, Usmani HA, Khan MI, Blakely EL, Taylor RW, Vassallo G, et al. Bilateral paediatric optic neuropathy precipitated by vitamin B12 deficiency and a novel mitochondrial DNA mutation. *Int Ophthalmol.* 2013;33:687-90.

391. Gopinath B, Flood VM, Rochtchina E, Wang JJ, Mitchell P. Homocysteine, folate, vitamin B-12, and 10-y incidence of age-related macular degeneration. *Am J Clin Nutr.* 2013;98:129-35.
392. Zemleni J, Wijeratne SSK, Kuroishi T. Biotin. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 359-74.
393. Mock DM, Malik MI. Distribution of biotin in human plasma: most of the biotin is not bound to protein. *Am J Clin Nutr.* 1992;56:427-32.
394. Mock NI, Malik MI, Stumbo PJ, Bishop WP, Mock DM. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased biotin status in experimental biotin deficiency. *Am J Clin Nutr.* 1997;65:951-8.
395. Mock DM. Biotin status: which are valid indicators and how do we know? *J Nutr.* 1999;129:498S-503S.
396. Mock NI, Mock DM. Biotin deficiency in rats: disturbances of leucine metabolism are detectable early. *J Nutr.* 1992;122:1493-9.
397. Lewis B, Rathman S, McMahon R. Dietary biotin intake modulates the pool of free and protein-bound biotin in rat liver. *J Nutr.* 2001;131:2310-5.
398. Mock DM, Stadler DD, Stratton SL, Mock NI. Biotin status assessed longitudinally in pregnant women. *J Nutr.* 1997;127:710-6.
399. Krause KH, Bonjour JP, Berlit P, Kynast G, Schmidt-Gayk H, Schellenberg B. Effect of long-term treatment with antiepileptic drugs on the vitamin status. *Drug Nutr Interact.* 1988;5:317-43.
400. Rathman SC, Gregory JF, 3rd, McMahon RJ. Pharmacological biotin supplementation maintains biotin status and function in rats administered dietary carbamazepine. *J Nutr.* 2003;133:2857-62.
401. Maeda Y, Kawata S, Inui Y, Fukuda K, Igura T, Matsuzawa Y. Biotin deficiency decreases ornithine transcarbamylase activity and mRNA in rat liver. *J Nutr.* 1996;126:61-6.
402. Borboni P, Magnaterra R, Rabini RA, Staffolani R, Porzio O, Sesti G, et al. Effect of biotin on glucokinase activity, mRNA expression and insulin release in cultured beta-cells. *Acta Diabetol.* 1996;33:154-8.
403. Dakshinamurti K, Li W. Transcriptional regulation of liver phosphoenolpyruvate carboxykinase by biotin in diabetic rats. *Mol Cell Biochem.* 1994;132:127-32.
404. Miller JW, Rucker RB. Pantothenic acid. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 375-90.
405. Fry PC, Fox HM, Tao HG. Metabolic response to a pantothenic acid deficient diet in humans. *J Nutr Sci Vitaminol (Tokyo).* 1976;22:339-46.
406. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, et al. Dietary changes favorably affect bone remodeling in older adults. *J Am Diet Assoc.* 1999;99:1228-33.
407. Weaver CM, Heaney RP. Calcium. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease.* 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 141-55.
408. Sauberlich H. Laboratory tests for the assessment of nutritional status. Boca Raton: CRC Press; 1999.
409. Nordin BE. Calcium homeostasis. *Clin Biochem.* 1990;23:3-10.
410. Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press; 1997.
411. Morgulis S. Chemical changes in the blood during fasting and subsequent refeeding. II. Inorganic constituents. *Am J Physiol.* 1928;84:350-62.
412. Oganov VS, Rakhmanov AS, Novikov VE, Zatsepin ST, Rodionova SS, Cann C. The state of human bone tissue during space flight. *Acta Astronaut.* 1991;23:129-33.
413. Smith MC, Jr, Rambaut PC, Vogel JM, Whittle MW. Bone mineral measurement—experiment M078. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377).* Washington, DC: National Aeronautics and Space Administration; 1977. p. 183-90.
414. Stupakov GP, Kazeykin VS, Kozlovskiy AP, Korolev VV. [Evaluation of changes in human axial skeletal bone structures during long-term spaceflights]. *Kosm Biol Aviakosm Med.* 1984;18(2):33-7.
415. Whedon GD. Disuse osteoporosis: physiological aspects. *Calcif Tissue Int.* 1984;36:S146-50.
416. Rambaut PC, Goode AW. Skeletal changes during space flight. *Lancet.* 1985;2:1050-2.
417. LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, et al. Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact.* 2000;1:157-60.
418. Sibonga JD. Spaceflight-induced bone loss: is there an osteoporosis risk? *Curr Osteoporos Rep.* 2013;11:92-8.

## *Risk Factor of Inadequate Nutrition*

419. Sibonga JD, Cavanagh PR, Lang TF, LeBlanc AD, Schneider VS, Shackelford LC, et al. Adaptation of the skeletal system during long-duration spaceflight. *Clin Rev Bone Miner Metab.* 2008;5:249-61.
420. Sibonga JD, Evans HJ, Sung HG, Spector ER, Lang TF, Oganov VS, et al. Recovery of spaceflight-induced bone loss: bone mineral density after long-duration missions as fitted with an exponential function. *Bone.* 2007;41:973-8.
421. Smith SM, Abrams SA, Davis-Street JE, Heer M, O'Brien KO, Wastney ME, et al. Fifty years of human space travel: implications for bone and calcium research. *Annu Rev Nutr.* 2014;34:377-400.
422. Holick MF. Microgravity-induced bone loss - will it limit human space exploration? *Lancet.* 2000;355:1569-70.
423. Carmeliet G, Vico L, Bouillon R. Space flight: a challenge for normal bone homeostasis. *Crit Rev Eukaryot Gene Expr.* 2001;11:131-44.
424. LeBlanc AD, Spector ER, Evans HJ, Sibonga JD. Skeletal responses to space flight and the bed rest analog: a review. *J Musculoskelet Neuronal Interact.* 2007;7:33-47.
425. Weaver CM, LeBlanc A, Smith SM. Calcium and related nutrients in bone metabolism. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models.* Boca Raton, FL: CRC Press; 2000. p. 179-201.
426. Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, Rehaillia M, et al. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. *Lancet.* 2000;355:1607-11.
427. Whedon GD, Lutwak L, Reid J, Rambaut PC, Whittle MW, Smith MC, et al. Mineral and nitrogen metabolic studies on Skylab orbital space flights. *Trans Assoc Am Physicians.* 1974;87:95-110.
428. Whedon G, Lutwak L, Rambaut P, Whittle M, Reid J, Smith M, et al. Mineral and nitrogen balance study observations: the second manned Skylab mission. *Aviat Space Environ Med.* 1976;47:391-6.
429. Rambaut PC, Johnston RS. Prolonged weightlessness and calcium loss in man. *Acta Astronaut.* 1979;6:1113-22.
430. Whedon G, Heaney R. Effects of physical inactivity, paralysis and weightlessness on bone growth. In: Hall B, editor. *Bone.* Boca Raton: CRC Press; 1993. p. 57-77.
431. Smith SM, Nillen JL, LeBlanc A, Lipton A, Demers LM, Lane HW, et al. Collagen cross-link excretion during space flight and bed rest. *J Clin Endocrinol Metab.* 1998;83:3584-91.
432. Caillot-Augusseau A, Lafage-Proust MH, Soler C, Pernod J, Dubois F, Alexandre C. Bone formation and resorption biological markers in cosmonauts during and after a 180-day space flight (Euromir 95). *Clin Chem.* 1998;44:578-85.
433. Collet P, Uebelhart D, Vico L, Moro L, Hartmann D, Roth M, et al. Effects of 1- and 6-month spaceflight on bone mass and biochemistry in two humans. *Bone.* 1997;20:547-51.
434. Leach CS, Rambaut PC, Di Ferrante N. Amino aciduria in weightlessness. *Acta Astronaut.* 1979;6:1323-33.
435. Baecker N, Frings-Meuthen P, Smith SM, Heer M. Short-term high dietary calcium intake during bedrest has no effect on markers of bone turnover in healthy men. *Nutrition.* 2010;26:522-7.
436. LeBlanc AD, Schneider VS, Evans HJ, Engelbretson DA, Krebs JM. Bone mineral loss and recovery after 17 weeks of bed rest. *J Bone Miner Res.* 1990;5:843-50.
437. Zerwekh JE, Ruml LA, Gottschalk F, Pak CY. The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. *J Bone Miner Res.* 1998;13:1594-601.
438. Vico L, Chappard D, Alexandre C, Palle S, Minaire P, Riffat G, et al. Effects of a 120 day period of bed-rest on bone mass and bone cell activities in man: attempts at countermeasure. *Bone Miner.* 1987;2:383-94.
439. LeBlanc A, Schneider V, Spector E, Evans H, Rowe R, Lane H, et al. Calcium absorption, endogenous excretion, and endocrine changes during and after long-term bed rest. *Bone.* 1995;16 (4 suppl):301S-4S.
440. Heer M, Baecker N, Mika C, Boese A, Gerzer R. Immobilization induces a very rapid increase in osteoclast activity. *Acta Astronaut.* 2005;57:31-6.
441. Arnaud SB, Sherrard DJ, Maloney N, Whalen RT, Fung P. Effects of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. *Aviat Space Environ Med.* 1992;63:14-20.
442. LeBlanc A, Schneider V, Krebs J, Evans H, Jhingran S, Johnson P. Spinal bone mineral after 5 weeks of bed rest. *Calcif Tissue Int.* 1987;41:259-61.
443. Deitrick JE, Whedon GD, Shorr E. Effects of immobilization upon various metabolic and physiologic functions of normal men. *Am J Med.* 1948;4:3-36.
444. Hwang TIS, Hill K, Schneider V, Pak CYC. Effect of prolonged bedrest on the propensity for renal stone formation. *J Clin Endocrinol Metab.* 1988;66:109-12.

## *Risk Factor of Inadequate Nutrition*

445. Donaldson CL, Hulley SB, Vogel JM, Hattner RS, Bayers JH, McMillan DE. Effect of prolonged bed rest on bone mineral. *Metabolism*. 1970;19:1071-84.
446. Whedon G, Lutwak L, Rambaut P, Whittle M, Leach C, Reid J, et al. Effect of weightlessness on mineral metabolism; metabolic studies on Skylab orbital flights. *Calcif Tissue Res*. 1976;21 Suppl:423-30.
447. Arnaud SB, Fung P, Popova IA, Morey-Holton ER, Grindeland RE. Circulating parathyroid hormone and calcitonin in rats after spaceflight. *J Appl Physiol*. 1992;73:169S-73S.
448. Jowsey J, Riggs BL, Goldsmith RS, Kelly PJ, Arnaud CD. Effects of prolonged administration of porcine calcitonin in postmenopausal osteoporosis. *J Clin Endocrinol Metab*. 1971;33:752-8.
449. Zorbas YG, Kakuris KK, Deogenov VA, Yerullis KB. Inadequacy of calcium supplements to normalize muscle calcium deficiency in healthy subjects during prolonged hypokinesia. *Nutrition*. 2008;24:217-23.
450. Elias AN, Gwinup G. Immobilization osteoporosis in paraplegia. *J Am Paraplegia Soc*. 1992;15:163-70.
451. Meythaler JM, Tuel SM, Cross LL. Successful treatment of immobilization hypercalcemia using calcitonin and etidronate. *Arch Phys Med Rehabil*. 1993;74:316-9.
452. Klein L, van der Noort S, DeJak JJ. Sequential studies of urinary hydroxyproline and serum alkaline phosphatase in acute paraplegia. *Med Serv J Can*. 1966;22:524-33.
453. Naftchi NE, Viau AT, Sell GH, Lowman EW. Mineral metabolism in spinal cord injury. *Arch Phys Med Rehabil*. 1980;61:139-42.
454. Stewart AF, Akler M, Byers CM, Segre GV, Broadus AE. Calcium homeostasis in immobilization: an example of resorptive hypercalciuria. *N Engl J Med*. 1982;306:1136-40.
455. Minaire P, Meunier P, Edouard C, Bernard J, Courpron P, Bourret J. Quantitative histological data on disuse osteoporosis: comparison with biological data. *Calcif Tissue Int*. 1974;17:57-73.
456. Grigoriev AI, Oganov VS, Bakulin AV, Poliakov VV, Voronin LI, Morgun VV, et al. [Clinical and physiological evaluation of bone changes among astronauts after long-term space flights]. *Aviakosm Ekolog Med*. 1998;32:21-5.
457. Androjna C, McCabe NP, Cavanagh PR, Midura RJ. Effects of spaceflight and skeletal unloading on bone fracture healing. *Clinic Rev Bone Miner Metab*. 2014;10:61-70.
458. Tilton FE, Degioanni JJC, Schneider VS. Long-term follow-up of Skylab bone demineralization. *Aviat Space Environ Med*. 1980;51:1209-13.
459. Morey-Holton E, Whalen R, Arnaud S, Van Der Meulen M. The skeleton and its adaptation to gravity. In: Fregly M, Blatteis C, editors. *Environmental physiology*. New York: Oxford University Press; 1996. p. 691-719.
460. Heer M, Kamps N, Biener C, Korr C, Boerger A, Zittermann A, et al. Calcium metabolism in microgravity. *Eur J Med Res*. 1999;4:357-60.
461. Arnaud SB, Schneider VS, Morey-Holton E. Effects of inactivity on bone and calcium metabolism. In: Sandler H, Vernikos J, editors. *Inactivity: physiological effects*. Orlando, FL: Academic Press, Inc.; 1986. p. 49-76.
462. Schneider VS, McDonald J. Skeletal calcium homeostasis and countermeasures to prevent disuse osteoporosis. *Calcif Tissue Int*. 1984;36 Suppl 1:S151-44.
463. Smith SM, Heer M. Calcium and bone metabolism during space flight. *Nutrition*. 2002;18:849-52.
464. Breen TL, Shane E. Prolonged hypocalcemia after treatment with zoledronic acid in a patient with prostate cancer and vitamin D deficiency. *J Clin Oncol*. 2004;22:1531-2.
465. Peter R, Mishra V, Fraser WD. Severe hypocalcaemia after being given intravenous bisphosphonate. *BMJ*. 2004;328:335-6.
466. Berenson J, Hirschberg R. Safety and convenience of a 15-minute infusion of zoledronic acid. *Oncologist*. 2004;9:319-29.
467. Breay S. Hypocalcaemia after intravenous bisphosphonate: read the product information first. *BMJ*. 2004;328:1439; author reply -40.
468. Knochel JP. Phosphorus. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 157-67.
469. Davies KM, Rafferty K, Heaney RP. Determinants of endogenous calcium entry into the gut. *Am J Clin Nutr*. 2004;80:919-23.
470. Lotz M, Zisman E, Bartter FC. Evidence for a phosphorus-depletion syndrome in man. *N Engl J Med*. 1968;278:409-15.
471. Jara A, Lee E, Stauber D, Moatamed F, Felsenfeld AJ, Kleeman CR. Phosphate depletion in the rat: effect of bisphosphonates and the calcemic response to PTH. *Kidney Int*. 1999;55:1434-43.

472. Milne DB. Trace elements. In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia, PA: WB Saunders Company; 1994. p. 1317-347.
473. Shils ME. Magnesium. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 169-92.
474. Leach CS. Biochemical and hematologic changes after short-term space flight. *Microgravity Q.* 1992;2:69-75.
475. Prokhonchukov AA, Zaitsev VP, Shakhunov BA, Zhizhina NA, Kolesnik AG. [Effect of space flight on the concentration of sodium, copper, manganese and magnesium in the bones of the skeleton]. *Patol Fiziol Eksp Ter.* 1978;65-70.
476. Aggett PJ. Iron. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 9th ed. Washington, DC: International Life Sciences Institute; 2010. p. 506-20.
477. Fairbanks VF. Iron in medicine and nutrition. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 193-221.
478. Milne DB, Gallagher SK, Nielsen FH. Response of various indices of iron status to acute iron depletion produced in menstruating women by low iron intake and phlebotomy. *Clin Chem.* 1990;36:487-91.
479. Prieto J, Barry M, Sherlock S. Serum ferritin in patients with iron overload and with acute and chronic liver disease. *Gastroenterology.* 1975;68:525-33.
480. Viteri FE, Torun B. Anaemia and physical work capacity. *Clin Hematol.* 1974;3:609-26.
481. Lozoff B. Behavioral alterations in iron deficiency. *Adv Pediatr.* 1988;35:331-59.
482. Dallman PR. Iron deficiency and the immune response. *Am J Clin Nutr.* 1987;46:329-34.
483. Yip R, Dallman PR. Iron. In: Ziegler EE, Filer LJ, Jr., editors. Present knowledge in nutrition. 7th ed. Washington, DC: International Life Sciences Institute; 1996. p. 277-92.
484. Cook JD. Adaptation in iron metabolism. *Am J Clin Nutr.* 1990;51:301-8.
485. Finch CA, Huebers HA. Perspectives in iron metabolism. *N Engl J Med.* 1982;306:1520-8.
486. Dao MC, Meydani SN. Iron biology, immunology, aging, and obesity: four fields connected by the small peptide hormone hepcidin. *Adv Nutr.* 2013;4:602-17.
487. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet.* 2006;367:133-43.
488. Doherty CP. Host-pathogen interactions: the role of iron. *J Nutr.* 2007;137:1341-4.
489. Alfrey CP, Rice L, Udden MM, Driscoll TB. Neocytolysis: physiological down-regulator of red-cell mass. *Lancet.* 1997;349:1389-90.
490. Alfrey CP, Udden MM, Leach-Huntoon C, Driscoll T, Pickett MH. Control of red blood cell mass in spaceflight. *J Appl Physiol.* 1996;81:98-104.
491. Johnson PC. The erythropoietic effects of weightlessness. In: Dunn CDR, editor. Current concepts in erythropoiesis. New York: John Wiley & Sons Ltd.; 1983. p. 279-300.
492. Udden MM, Driscoll TB, Pickett MH, Leach-Huntoon CS, Alfrey CP. Decreased production of red blood cells in human subjects exposed to microgravity. *J Lab Clin Med.* 1995;125:442-9.
493. Smith SM. Red blood cell and iron metabolism during space flight. *Nutrition.* 2002;18:864-6.
494. Smith SM, Davis-Street JE, Fontenot TB, Lane HW. Assessment of a portable clinical blood analyzer during space flight. *Clin Chem.* 1997;43:1056-65.
495. Leach CS, Rambaut PC. Biochemical observations of long duration manned orbital spaceflight. *J Am Med Womens Assoc.* 1975;30:153-72.
496. Convertino VA. Clinical aspects of the control of plasma volume at microgravity and during return to one gravity. *Med Sci Sports Exerc.* 1996;28:S45-52.
497. Lane HW, Alfrey CP, Driscoll TB, Smith SM, Nyquist LE. Control of red blood cell mass during spaceflight. *J Gravitational Physiol.* 1996;3:87-8.
498. Zwart SR, Morgan JL, Smith SM. Iron status and its relations with oxidative damage and bone loss during long-duration space flight on the International Space Station. *Am J Clin Nutr.* 2013;98:217-23.
499. Beilby J, Olynyk J, Ching S, Prins A, Swanson N, Reed W, et al. Transferrin index: an alternative method for calculating the iron saturation of transferrin. *Clin Chem.* 1992;38:2078-81.
500. Mendes JF, Arruda SF, Siqueira EM, Ito MK, Silva EF. Iron status and oxidative stress biomarkers in adults: a preliminary study. *Nutrition.* 2009;25:379-84.
501. Tuomainen TP, Loft S, Nyssonen K, Punnonen K, Salonen JT, Poulsen HE. Body iron is a contributor to oxidative damage of DNA. *Free Radic Res.* 2007;41:324-8.

502. Syrovatka P, Kraml P, Potockova J, Fialova L, Vejrazka M, Crkovska J, et al. Relationship between increased body iron stores, oxidative stress and insulin resistance in healthy men. *Ann Nutr Metab.* 2009;54:268-74.
503. Dunn CDR, Lange RD, Kimzey SL, Johnson PC, Leach CS. Serum erythropoietin titers during prolonged bedrest; relevance to the "anaemia" of space flight. *Eur J Appl Physiol.* 1984;52:178-82.
504. Rice L, Ruiz W, Driscoll T, Whitley CE, Tapia R, Hachey DL, et al. Neocytolysis on descent from altitude: a newly recognized mechanism for the control of red cell mass. *Ann Intern Med.* 2001;134:652-6.
505. Britton RS, Leicester KL, Bacon BR. Iron toxicity and chelation therapy. *Int J Hematol.* 2002;76:219-28.
506. Gleis M, Latunde-Dada GO, Klinder A, Becker TW, Hermann U, Voigt K, et al. Iron-overload induces oxidative DNA damage in the human colon carcinoma cell line HT29 clone 19A. *Mutat Res.* 2002;519:151-61.
507. Zwart SR, Kala G, Smith SM. Body iron stores and oxidative damage in humans increased during and after a 10- to 12-day undersea dive. *J Nutr.* 2009;139:90-5.
508. Bader N, Boser-Westphal A, Koch A, Mueller MJ. Influence of vitamin C and E supplementation on oxidative stress induced by hyperbaric oxygen in healthy men. *Ann Nutr Metab.* 2006;50:173-6.
509. Rocco M, Antonelli M, Letizia V, Alampi D, Spadetta G, Passariello M, et al. Lipid peroxidation, circulating cytokine and endothelin 1 levels in healthy volunteers undergoing hyperbaric oxygenation. *Minerva Anesthesiol.* 2001;67:393-400.
510. Alleva R, Nasole E, Di Donato F, Borghi B, Neuzil J, Tomasetti M. alpha-Lipoic acid supplementation inhibits oxidative damage, accelerating chronic wound healing in patients undergoing hyperbaric oxygen therapy. *Biochem Biophys Res Commun.* 2005;333:404-10.
511. Djurhuus R, Segadal K, Svardal AM. Glutathione in blood cells decreases without DNA breaks after a simulated saturation dive to 250 msw. *Aviat Space Environ Med.* 2006;77:597-604.
512. Kim BJ, Ahn SH, Bae SJ, Kim EH, Lee SH, Kim HK, et al. Iron overload accelerates bone loss in healthy postmenopausal women and middle-aged men: a 3-year retrospective longitudinal study. *J Bone Miner Res.* 2012;27:2279-90.
513. He X, Hahn P, Iacovelli J, Wong R, King C, Bhisitkul R, et al. Iron homeostasis and toxicity in retinal degeneration. *Prog Retin Eye Res.* 2007;26:649-73.
514. Morgan JL, Ritchie LE, Crucian BE, Theriot C, Wu H, Sams C, et al. Increased dietary iron and radiation in rats promote oxidative stress, induce localized and systemic immune system responses, and alter colon mucosal environment. *FASEB J.* 2014;28:1486-98.
515. Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and the risk of coronary heart disease. *N Engl J Med.* 1994;330:1119-24.
516. Ascherio A, Willett WC. Are body iron stores related to the risk of coronary heart disease? (Editorial). *N Engl J Med.* 1994;330:1152-4.
517. Sullivan JL. Stored iron and ischemic heart disease: empirical support for a new paradigm (Editorial Comment). *Circulation.* 1992;86:1036-7.
518. Lauffer RB. Iron stores and the international variation in mortality from coronary artery disease. *Lancet.* 1991;2:1288-9.
519. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation.* 1992;86:803-11.
520. Sullivan JL. The iron paradigm of ischemic heart disease. *Am Heart J.* 1989;117:1177-88.
521. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakulinen T. Body iron stores and risk of cancer. *Int J Cancer.* 1994;56:379-82.
522. Mainous AG, 3rd, Wells BJ, Koopman RJ, Everett CJ, Gill JM. Iron, lipids, and risk of cancer in the Framingham Offspring cohort. *Am J Epidemiol.* 2005;161:1115-22.
523. Schreiber WE. Iron, porphyrin, and bilirubin metabolism. In: Kaplan LA, Pesce AJ, editors. *Clinical chemistry: theory, analysis, and correlation.* St. Louis, MO: Mosby-Year Books, Inc.; 1996. p. 696-715.
524. Wilson JW, Ott CM, Honer zu Bentrup K, Ramamurthy R, Quick L, Porwollik S, et al. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proc Natl Acad Sci U S A.* 2007;104:16299-304.
525. Payne SM. Iron and virulence in the family Enterobacteriaceae. *Crit Rev Microbiol.* 1988;16:81-111.
526. Prohaska JR. Copper. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition.* 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 540-53.
527. Davis AT, Franz FP, Courtney DA, Ullrey DE, Scholten DJ, Dean RE. Plasma vitamin and mineral status in home parenteral nutrition patients. *JPEN J Parenter Enteral Nutr.* 1987;11:480-5.

## *Risk Factor of Inadequate Nutrition*

528. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes: a risk assessment model for establishing upper intake levels for nutrients. Washington, DC: National Academies Press; 1998.
529. Higuchi S, Higashi A, Nakamura T, Matsuda I. Nutritional copper deficiency in severely handicapped patients on a low copper enteral diet for a prolonged period: estimation of the required dose of dietary copper. *J Pediatr Gastroenterol Nutr.* 1988;7:583-7.
530. Milne DB. Copper intake and assessment of copper status. *Am J Clin Nutr.* 1998;67:1041S-5S.
531. Krebs JM, Schneider VS, LeBlanc AD, Kuo MC, Spector E, Lane HW. Zinc and copper balances in healthy adult males during and after 17 wk of bed rest. *Am J Clin Nutr.* 1993;58:897-901.
532. Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. *J Nutr Health Aging.* 2006;10:377-85.
533. Taylor GR, Konstantinova I, Sonnenfeld G, Jennings R. Changes in the immune system during and after spaceflight. *Adv Space Biol Med.* 1997;6:1-32.
534. Borchers AT, Keen CL, Gershwin ME. Microgravity and immune responsiveness: implications for space travel. *Nutrition.* 2002;18:889-98.
535. Tipton CM, Greenleaf JE, Jackson CG. Neuroendocrine and immune system responses with spaceflights. *Med Sci Sports Exerc.* 1996;28:988-98.
536. Crucian BE, Stowe RP, Pierson DL, Sams CF. Immune system dysregulation following short- vs long-duration spaceflight. *Aviat Space Environ Med.* 2008;79:835-43.
537. Devine A, Hodgson JM, Dick IM, Prince RL. Tea drinking is associated with benefits on bone density in older women. *Am J Clin Nutr.* 2007;86:1243-7.
538. Freeland-Graves J, Llanes C. Models to study manganese deficiency. In: Klimis-Tavantzis DJ, editor. *Manganese in health and disease.* Boca Raton, FL: CRC Press; 1994. p. 59-86.
539. Maheshwari UR, McDonald JT, Schneider VS, Brunetti AJ, Leybin L, Newbrun E, et al. Fluoride balance studies in ambulatory healthy men with and without fluoride supplements. *Am J Clin Nutr.* 1981;34:2679-84.
540. Riggs BL, Jowsey J. Treatment of osteoporosis with fluoride. *Semin Drug Treat.* 1972;2:27-33.
541. Riggs BL, Jowsey J. Treatment of Paget's disease with fluoride. *Semin Drug Treat.* 1972;2:65-8.
542. Maheshwari UR, Schneider VS, McDonald JT, Brunetti AJ, Leybin L, Newbrun E, et al. Fluoride balance studies in healthy men during bed rest with and without a fluoride supplement. *Am J Clin Nutr.* 1982;36:211-8.
543. King JC, Keen CL. Zinc. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease.* 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1999. p. 223-39.
544. Wada L, King JC. Effect of low zinc intakes on basal metabolic rate, thyroid hormones and protein utilization in adult men. *J Nutr.* 1986;116:1045-53.
545. Krebs JM, Schneider VS, LeBlanc AD. Zinc, copper, and nitrogen balances during bed rest and fluoride supplementation in healthy adult males. *Am J Clin Nutr.* 1988;47:509-14.
546. Cann CE, Adachi RR. Bone resorption and mineral excretion in rats during spaceflight. *Am J Physiol.* 1983;13:R327-31.
547. Kondrashov V, Rothenberg SJ, Chettle D, Zerwekh J. Evaluation of potentially significant increase of lead in the blood during long-term bed rest and space flight. *Physiol Meas.* 2005;26:1-12.
548. Kondrashov VS. Cosmonauts and lead: resorption and increased blood lead levels during long term space flight. *J Med Toxicol.* 2006;2:172-3.
549. van den Brandt PA, Zeegers MP, Bode P, Goldbohm RA. Toenail selenium levels and the subsequent risk of prostate cancer: a prospective cohort study. *Cancer Epidemiol Biomarkers Prev.* 2003;12:866-71.
550. National Aeronautics and Space Administration Johnson Space Center. Nutritional status assessment for extended-duration space flight. Report No.: JSC-28566, Revision 1. Houston, TX: Lyndon B. Johnson Space Center; 1999.
551. Naeije R, Vanhaelst L, Golstein J. Pituitary-thyroid axis during short term, mild and severe, iodine depletion in the rat. *Horm Metab Res.* 1978;10:521-5.
552. National Research Council. Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: The National Academy Press; 2000.
553. Sauer RL, Janik DS, Thorstenson YR. Medical effects of iodine disinfection products in spacecraft water. SAE technical report no. 871490. Warrendale, PA: Society of Automotive Engineers 1987.
554. National Aeronautics and Space Administration. Medical effects of iodine: proceedings of NASA/JSC conference. Houston, TX: Lyndon B. Johnson Space Center 1998. Report No.: JSC 28379.

555. McMonigal KA, Braverman LE, Dunn JT, Stanbury JB, Wear ML, Hamm PB, et al. Thyroid function changes related to use of iodinated water in the U.S. Space Program. *Aviat Space Environ Med.* 2000;71:1120-5.
556. McMonigal K, Sauer RL, Smith SM, Pattinson T, Gillman PL, Davis-Street JE, et al. Physiological effects of iodinated water on thyroid function. In: Lane HW, Sauer RL, Feedback DL, editors. *Isolation: NASA experiments in closed-environment living.* San Diego: Univelt, Inc.; 2002. p. 369-95.
557. National Research Council, Committee on Life Sciences, Food and Nutrition Board, Subcommittee on the Tenth Edition of the RDAs. *Recommended Dietary Allowances, 10th ed.* Washington, DC: National Academy Press; 1989.
558. Brown RO, Forloines-Lynn S, Cross RE, Heizer WD. Chromium deficiency after long-term total parenteral nutrition. *Dig Dis Sci.* 1986;31:661-4.
559. Anderson RA, Polansky MM, Bryden NA, Canary JJ. Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutr.* 1991;54:909-16.
560. Mader TH, Gibson CR, Pass AF, Kramer LA, Lee AG, Fogarty J, et al. Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. *Ophthalmology.* 2011;118:2058-69.
561. Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *J Bone Miner Res.* 2004;19:1006-12.
562. Loehr JA, Lee SM, English KL, Sibonga J, Smith SM, Spiering BA, et al. Musculoskeletal adaptations to training with the advanced resistive exercise device. *Med Sci Sports Exerc.* 2011;43:146-56.
563. Ploutz-Snyder L, Bloomfield S, Smith SM, Hunter SK, Templeton KE, Bembien D. Effects of sex and gender on adaptation to space: Musculoskeletal health. *J Womens Health.* 2014;23:963-6.
564. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol.* 2004;97:119-29.
565. Tsay J, Yang Z, Ross FP, Cunningham-Rundles S, Lin H, Coleman R, et al. Bone loss caused by iron overload in a murine model: importance of oxidative stress. *Blood.* 2010;116:2582-9.
566. Yuen E, Morgan JLL, Zwart SR, Gonzales E, Camp K, Macias BR, et al. High iron diet and radiation exposure induce oxidative stress and reduce bone density (Abstr. 286). *Am Soc Bone Miner Res; Minneapolis, MN2012.*
567. Hamilton SA, Pecaunt MJ, Gridley DS, Travis ND, Bandstra ER, Willey JS, et al. A murine model for bone loss from therapeutic and space-relevant sources of radiation. *J Appl Physiol.* 2006;101:789-93.
568. Lloyd SA, Bandstra ER, Willey JS, Riffle SE, Tirado-Lee L, Nelson GA, et al. Effect of proton irradiation followed by hindlimb unloading on bone in mature mice: a model of long-duration spaceflight. *Bone.* 2012;51:756-64.
569. Chuenchitra T, Chaitaveep P, Sukwit S, Dettrairat S, Tabprasit S, Srisurapanon S, et al. Cytokine profiles in HIV-1 subtype CRF01\_AE infected individuals with different rates of diseases progression: a multiplex immunoassay. *J Med Assoc Thai.* 2012;95 Suppl 5:S116-23.
570. Khan IH, Krishnan VV, Ziman M, Janatpour K, Wun T, Luciw PA, et al. A comparison of multiplex suspension array large-panel kits for profiling cytokines and chemokines in rheumatoid arthritis patients. *Cytometry B Clin Cytom.* 2009;76:159-68.
571. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. *J Clin Oncol.* 2011;29:1356-63.
572. Szodoray P, Alex P, Brun JG, Centola M, Jonsson R. Circulating cytokines in primary Sjogren's syndrome determined by a multiplex cytokine array system. *Scand J Immunol.* 2004;59:592-9.
573. Bon JM, Zhang Y, Duncan SR, Pilewski JM, Zaltonis D, Zeevi A, et al. Plasma inflammatory mediators associated with bone metabolism in COPD. *COPD.* 2010;7:186-91.
574. Chen KS, Wang PH, Yang SF, Lin DB, Lin YJ, Kuo DY, et al. Significant elevation of a Th2 cytokine, interleukin-10, in pelvic inflammatory disease. *Clin Chem Lab Med.* 2008;46:1609-16.
575. Crucian B, Sams C. Immune system dysregulation during spaceflight: clinical risk for exploration-class missions. *J Leukoc Biol.* 2009;86:1017-8.
576. Crucian B, Stowe R, Mehta S, Uchakin P, Quiriarte H, Pierson D, et al. Immune system dysregulation occurs during short duration spaceflight on board the space shuttle. *J Clin Immunol.* 2013;33:456-65.

## *Risk Factor of Inadequate Nutrition*

577. Crucian BE, Zwart SR, Mehta S, Uchakin P, Quiariarte HD, Pierson D, et al. Plasma cytokine concentrations indicate that in vivo hormonal regulation of immunity is altered during long-duration spaceflight. *J Interferon Cytokine Res.* 2014;34:778-86.
578. Matsumoto A, Storch KJ, Stolfi A, Mohler SR, Frey MA, Stein TP. Weight loss in humans in space. *Aviat Space Environ Med.* 2011;82:615-21.
579. Smith DC, Kaufman KA. Space linear acceleration mass measurement device (SLAMMD) for the human research facility (HRF) (Report #981652). Warrendale, PA: SAE International 1998.
580. Sarychev VA, Sazonov VV, Zlatorunsky AS, Khlopina SF, Egorov AD, Somov VI. Device for mass measurement under zero-gravity conditions. *Acta Astronaut.* 1980;7:719-30.
581. Wimalawansa SM, Chapa MT, Wei JN, Westlund KN, Quast MJ, Wimalawansa SJ. Reversal of weightlessness-induced musculoskeletal losses with androgens: quantification by MRI. *J Appl Physiol.* 1999;86:1841-6.
582. Zachwieja JJ, Smith SR, Lovejoy JC, Rood JC, Windhauser MM, Bray GA. Testosterone administration preserves protein balance but not muscle strength during 28 days of bed rest. *J Clin Endocrinol Metab.* 1999;84:207-12.
583. Smith SM, Heer M, Wang Z, Huntoon CL, Zwart SR. Long-duration space flight and bed rest effects on testosterone and other steroids. *J Clin Endocrinol Metab.* 2012;97:270-8. Erratum JCEM 97:3390. 2012.
584. McBarron JW, 2nd. U.S. prebreathe protocol. *Acta Astronaut.* 1994;32:75-8.
585. Wender DF, Thulin GE, Smith GJ, Warshaw JB. Vitamin E affects lung biochemical and morphologic response to hyperoxia in the newborn rabbit. *Pediatr Res.* 1981;15:262-8.
586. Takahashi H, Kosaka N, Nakagawa S. alpha-Tocopherol protects PC12 cells from hyperoxia-induced apoptosis. *J Neurosci Res.* 1998;52:184-91.
587. Niki E. Interaction of ascorbate and alpha-tocopherol. *Ann N Y Acad Sci.* 1987;498:186-99.
588. Smith SM, Davis-Street J, Rice BL, Lane HW. Nutrition in space. *Nutrition Today.* 1997;32:6-12.
589. Hoshi T, Tian Y, Xu R, Heinemann SH, Hou S. Mechanism of the modulation of BK potassium channel complexes with different auxiliary subunit compositions by the omega-3 fatty acid DHA. *Proc Natl Acad Sci U S A.* 2013;110:4822-7.
590. Hoshi T, Wissuwa B, Tian Y, Tajima N, Xu R, Bauer M, et al. Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> channels. *Proc Natl Acad Sci U S A.* 2013;110:4816-21.
591. Polivkova Z, Smerak P, Demova H, Houska M. Antimutagenic effects of lycopene and tomato puree. *J Med Food.* 2010;13:1443-50.
592. Basu A, Imrhan V. Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials. *Eur J Clin Nutr.* 2007;61:295-303.
593. Nicastro HL, Dunn BK. Selenium and prostate cancer prevention: insights from the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *Nutrients.* 2013;5:1122-48.
594. Dunn BK, Richmond ES, Minasian LM, Ryan AM, Ford LG. A nutrient approach to prostate cancer prevention: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *Nutr Cancer.* 2010;62:896-918.
595. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA.* 2008;300:2123-33.
596. American Dietetic Association. Position of the American Dietetic Association: fortification and nutritional supplements. *J Am Diet Assoc.* 2005;105:1300-11.
597. Goodman GE, Thornquist MD, Balmes J, Cullen MR, Meyskens FL, Jr., Omenn GS, et al. The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. *J Natl Cancer Inst.* 2004;96:1743-50.
598. Bjelakovic G, Nikolova D, Gluud C. Antioxidant supplements and mortality. *Curr Opin Clin Nutr Metab Care.* 2014;17:40-4.
599. Bjelakovic G, Nikolova D, Gluud C. Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm? *PLoS One.* 2013;8:e74558.
600. Hardman J, Limbird L. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw Hill; 1996.

601. Guengerich FP, Miller GP, Hanna IH, Martin MV, Leger S, Black C, et al. Diversity in the oxidation of substrates by cytochrome P450 2D6: lack of an obligatory role of aspartate 301-substrate electrostatic bonding. *Biochemistry (Mosc)*. 2002;41:11025-34.
602. Utermohlen V. Diet, nutrition, and drug interactions. In: Shils ME, Olson JA, Shike M, Ross AC, editors. *Modern nutrition in health and disease*. 9th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1998.
603. Guengerich FP. Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. *Am J Clin Nutr*. 1995;61:651S-8S.
604. Bailey DG, Spence JD, Edgar B, Bayliff CD, Arnold JM. Ethanol enhances the hemodynamic effects of felodipine. *Clin Invest Med*. 1989;12:357-62.
605. Kane GC, Lipsky JJ. Drug-grapefruit juice interactions. *Mayo Clin Proc*. 2000;75:933-42.
606. Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, et al. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest*. 1997;99:2545-53.
607. Lundahl J, Regardh CG, Edgar B, Johnsson G. Relationship between time of intake of grapefruit juice and its effect on pharmacokinetics and pharmacodynamics of felodipine in healthy subjects. *Eur J Clin Pharmacol*. 1995;49:61-7.
608. Piver B, Berthou F, Dreano Y, Lucas D. Inhibition of CYP3A, CYP1A and CYP2E1 activities by resveratrol and other non volatile red wine components. *Toxicol Lett*. 2001;125:83-91.
609. Chan WK, Nguyen LT, Miller VP, Harris RZ. Mechanism-based inactivation of human cytochrome P450 3A4 by grapefruit juice and red wine. *Life Sci*. 1998;62:PL135-42.
610. Roby CA, Anderson GD, Kantor E, Dryer DA, Burstein AH. St John's Wort: effect on CYP3A4 activity. *Clin Pharmacol Ther*. 2000;67:451-7.
611. Fujita K. Food-drug interactions via human cytochrome P450 3A (CYP3A). *Drug Metabol Drug Interact*. 2004;20:195-217.
612. Fagan TC, Walle T, Oexmann MJ, Walle UK, Bai SA, Gaffney TE. Increased clearance of propranolol and theophylline by high-protein compared with high-carbohydrate diet. *Clin Pharmacol Ther*. 1987;41:402-6.
613. Saarem K, Pedersen JI. Sex differences in the hydroxylation of cholecalciferol and of 5 beta-cholestane-3 alpha, 7 alpha, 12 alpha-triol in rat liver. *Biochem J*. 1987;247:73-8.
614. Gupta RP, Hollis BW, Patel SB, Patrick KS, Bell NH. CYP3A4 is a human microsomal vitamin D 25-hydroxylase. *J Bone Miner Res*. 2004;19:680-8.
615. Schuster I, Egger H, Reddy GS, Vorisek G. Combination of vitamin D metabolites with selective inhibitors of vitamin D metabolism. *Recent Results Cancer Res*. 2003;164:169-88.
616. Pascussi JM, Robert A, Nguyen M, Walrant-Debray O, Garabedian M, Martin P, et al. Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. *J Clin Invest*. 2005;115:177-86.
617. Self TH, Chrisman CR, Baciewicz AM, Bronze MS. Isoniazid drug and food interactions. *Am J Med Sci*. 1999;317:304-11.
618. Shah SC, Sharma RK, Hemangini, Chitle AR. Rifampicin induced osteomalacia. *Tubercle*. 1981;62:207-9.
619. Goraya JS, Gupta PN, Gupta RK, Bahadur R, Parmar VR. Anticonvulsant induced osteomalacia. *Indian Pediatr*. 2000;37:325-9.
620. Chen H, Howald WN, Juchau MR. Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes. *Drug Metab Dispos*. 2000;28:315-22.
621. Roberts ES, Vaz AD, Coon MJ. Role of isozymes of rabbit microsomal cytochrome P-450 in the metabolism of retinoic acid, retinol, and retinal. *Mol Pharmacol*. 1992;41:427-33.
622. Force RW, Nahata MC. Effect of histamine H2-receptor antagonists on vitamin B12 absorption. *Ann Pharmacother*. 1992;26:1283-6.
623. Mowat C, McColl KE. Alterations in intragastric nitrite and vitamin C levels during acid inhibitory therapy. *Best Pract Res Clin Gastroenterol*. 2001;15:523-37.
624. Russell RM, Golner BB, Krasinski SD, Sadowski JA, Suter PM, Braun CL. Effect of antacid and H<sub>2</sub> receptor antagonists on the intestinal absorption of folic acid. *J Lab Clin Med*. 1988;112:458-63.
625. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*. 2008;88:1243-76.
626. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Lett*. 2012;327:48-60.

627. Li M, Gonon G, Buonanno M, Autsavapromporn N, de Toledo SM, Pain D, et al. Health risks of space exploration: targeted and nontargeted oxidative injury by high-charge and high-energy particles. *Antioxid Redox Signal*. 2014;20:1501-23.
628. Gernhardt ML, Jones JA, Scheuring RA, Abercromby AF, Tuxhorn JA, Norcross JR. Risk of compromised EVA performance and crew health due to inadequate EVA suit systems. In: McPhee JS, Charles JB, editors. *Human health and performance risks of space exploration missions: evidence reviewed by the NASA Human Research Program*. Washington, DC: Government Printing Office; 2009. p. 333-58.
629. Norcross J, Norsk P, Law J, Arias D, Conkin J, Perchonok M, et al. *Effects of the 8 psia / 32% O2 atmosphere on the human in the spaceflight environment*. Hanover, MD: National Aeronautics and Space Administration Center for AeroSpace Information; 2013.
630. Reid MB. Muscle fatigue: mechanisms and regulation. In: Sen CK, Packer L, Hänninen O, editors. *Handbook of oxidants and antioxidants in exercise*. Amsterdam: Elsevier Science B.V.; 2000. p. 599-630.
631. Upton AC. The biological effects of low-level ionizing radiation. *Sci Am*. 1982;246(2):41-9.
632. O'Neill CA, Stebbins CL, Bonigut S, Halliwell B, Longhurst JC. Production of hydroxyl radicals in contracting skeletal muscle of cats. *J Appl Physiol*. 1996;81:1197-206.
633. Reid MB, Khawli FA, Moody MR. Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *J Appl Physiol*. 1993;75:1081-7.
634. Essig DA, Nosek TM. Muscle fatigue and induction of stress protein genes: a dual function of reactive oxygen species? *Can J Appl Physiol*. 1997;22:409-28.
635. Lawler JM, Cline CC, Hu Z, Coast JR. Effect of oxidant challenge on contractile function of the aging rat diaphragm. *Am J Physiol*. 1997;272:E201-7.
636. Jones JA, Riggs PK, Yang TC, Pedemonte CH, Clarke MS, Feedback DL, et al. Ionizing radiation-induced bioeffects in space and strategies to reduce cellular injury and carcinogenesis. *Aviat Space Environ Med*. 2007;78:A67-78.
637. Kennedy AR, Ware JH, Guan J, Donahue JJ, Biaglow JE, Zhou Z, et al. Selenomethionine protects against adverse biological effects induced by space radiation. *Free Radic Biol Med*. 2004;36:259-66.
638. Little JB. Radiation carcinogenesis. *Carcinogenesis*. 2000;21:397-404.
639. Mao XW, Pecaut MJ, Stodieck LS, Ferguson VL, Bateman TA, Boussein M, et al. Spaceflight environment induces mitochondrial oxidative damage in ocular tissue. *Radiat Res*. 2013;180:340-50.
640. Rizzo AM, Corsetto PA, Montorfano G, Milani S, Zava S, Tavella S, et al. Effects of long-term space flight on erythrocytes and oxidative stress of rodents. *PLoS One*. 2012;7:e32361.
641. Baqai FP, Gridley DS, Slater JM, Luo-Owen X, Stodieck LS, Ferguson V, et al. Effects of spaceflight on innate immune function and antioxidant gene expression. *J Appl Physiol*. 2009;106:1935-42.
642. Gridley DS, Slater JM, Luo-Owen X, Rizvi A, Chapes SK, Stodieck LS, et al. Spaceflight effects on T lymphocyte distribution, function and gene expression. *J Appl Physiol* (1985). 2009;106:194-202.
643. Zezerov AE, Ivanova SM, Morukov BV, Ushakov AS. [Lipid peroxidation in the human blood during a 120-day period of anti-orthostatic hypokinesia]. *Kosm Biol Aviakosm Med*. 1989;23:28-33.
644. Pross HD, Casares A, Kiefer J. Induction and repair of DNA double-strand breaks under irradiation and microgravity. *Radiat Res*. 2000;153:521-5.
645. Kiefer J, Pross HD. Space radiation effects and microgravity. *Mutat Res*. 1999;430:299-305.
646. Hollander J, Gore M, Fiebig R, Mazzeo R, Ohishi S, Ohno H, et al. Spaceflight downregulates antioxidant defense systems in rat liver. *Free Radic Biol Med*. 1998;24:385-90.
647. Cocate PG, Natali AJ, de Oliveira A, Longo GZ, Alfenas Rde C, Peluzio Mdo C, et al. Fruit and vegetable intake and related nutrients are associated with oxidative stress markers in middle-aged men. *Nutrition*. 2014;30:660-5.
648. Moco S, Martin FP, Rezzi S. Metabolomics view on gut microbiome modulation by polyphenol-rich foods. *J Proteome Res*. 2012;11:4781-90.
649. Zeng H, Lazarova DL, Bordonaro M. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. *World J Gastrointest Oncol*. 2014;6:41-51.
650. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505:559-63.
651. Terry EN, Diamond AM. Selenium. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition*. 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 568-85.

## *Risk Factor of Inadequate Nutrition*

652. Klein EA, Thompson IM, Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 2011;306:1549-56.
653. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO. Antioxidants accelerate lung cancer progression in mice. *Sci Transl Med*. 2014;6:221ra15.
654. Johnston CS. Vitamin C. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. Present knowledge in nutrition. 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 248-60.
655. Sahni S, Hannan MT, Gagnon D, Blumberg J, Cupples LA, Kiel DP, et al. High vitamin C intake is associated with lower 4-year bone loss in elderly men. *J Nutr*. 2008;138:1931-8.
656. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? *Am J Clin Nutr*. 2000;71:142-51.
657. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. *Am J Clin Nutr*. 1997;65:1831-9.
658. Macdonald HM, Black AJ, Aucott L, Duthie G, Duthie S, Sandison R, et al. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. *Am J Clin Nutr*. 2008;88:465-74.
659. Lanham-New SA. The balance of bone health: tipping the scales in favor of potassium-rich, bicarbonate-rich foods. *J Nutr*. 2008;138:172S-7S.
660. Masse PG, Jougoux JL, Tranchant CC, Dossy J, Caissie M, Coburn SP. Enhancement of calcium/vitamin D supplement efficacy by administering concomitantly three key nutrients essential to bone collagen matrix for the treatment of osteopenia in middle-aged women: a one-year follow-up. *J Clin Biochem Nutr*. 2010;46:20-9.
661. Sahni S, Hannan MT, Gagnon D, Blumberg J, Cupples LA, Kiel DP, et al. Protective effect of total and supplemental vitamin C intake on the risk of hip fracture--a 17-year follow-up from the Framingham Osteoporosis Study. *Osteoporos Int*. 2009;20:1853-61.
662. Morton DJ, Barrett-Connor EL, Schneider DL. Vitamin C supplement use and bone mineral density in postmenopausal women. *J Bone Miner Res*. 2001;16:135-40.
663. Hall SL, Greendale GA. The relation of dietary vitamin C intake to bone mineral density: results from the PEPI study. *Calcif Tissue Int*. 1998;63:183-9.
664. Thompson J. Vitamins, minerals and supplements 5: overview of vitamin C. *Community Pract*. 2007;80:35-6.
665. Yoshida M, Takashima Y, Inoue M, Iwasaki M, Otani T, Sasaki S, et al. Prospective study showing that dietary vitamin C reduced the risk of age-related cataracts in a middle-aged Japanese population. *Eur J Nutr*. 2007;46:118-24.
666. Watters JL, Satia JA, Kupper LL, Swenberg JA, Schroeder JC, Switzer BR. Associations of antioxidant nutrients and oxidative DNA damage in healthy African-American and White adults. *Cancer Epidemiol Biomarkers Prev*. 2007;16:1428-36.
667. Morgan JL, Zwart SR, Heer M, Ploutz-Snyder R, Ericson K, Smith SM. Bone metabolism and nutritional status during 30-day head-down-tilt bed rest. *J Appl Physiol*. 2012;113:1519-29.
668. Vico L, Lafage-Proust MH, Alexandre C. Effects of gravitational changes on the bone system in vitro and in vivo. *Bone*. 1998;22(5 Suppl):95S.
669. Vogel JM, Whittle MW. Proceedings: Bone mineral content changes in the Skylab astronauts. *Am J Roentgenol Radium Ther Nucl Med*. 1976;126:1296-7.
670. Oganov VS, Grigor'ev AI, Voronin LI, Rakhmanov AS, Bakulin AV, Schneider VS, et al. [Bone mineral density in cosmonauts after flights lasting 4.5-6 months on the Mir orbital station]. *Aviakosm Ekolog Med*. 1992;26:20-4.
671. Zerwekh JE. Nutrition and renal stone disease in space. *Nutrition*. 2002;18:857-63.
672. Orwoll ES, Adler RA, Amin S, Binkley N, Lewiecki EM, Petak SM, et al. Skeletal health in long-duration astronauts: nature, assessment and management recommendations from the NASA bone summit. *J Bone Miner Res*. 2013;28:1243-55.
673. Seo H, Itoh T, Murata Y, Ohmori S, Kambe F, Mohri M, et al. Changes in urinary excretion of pyridinium cross-links during Spacelab-J. *Biol Sci Space*. 1997;11:321-6.
674. Zittermann A, Heer M, Caillot-Augusso A, Rettberg P, Scheld K, Drummer C, et al. Microgravity inhibits intestinal calcium absorption as shown by a stable strontium test. *Eur J Clin Invest*. 2000;30:1036-43.

675. Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986-2006). *Eur J Appl Physiol.* 2007;101:143-94.
676. Spector ER, Smith SM, Sibonga JD. Skeletal effects of long-duration head-down bed rest. *Aviat Space Environ Med.* 2009;80:A23-8.
677. Hantman DA, Vogel JM, Donaldson CL, Friedman R, Goldsmith RS, Hulley SB. Attempts to prevent disuse osteoporosis by treatment with calcitonin, longitudinal compression and supplementary calcium and phosphate. *J Clin Endocrinol Metab.* 1973;36:845-58.
678. Smith SM, Zwart SR, Heer MA, Baecker N, Evans HJ, Feiveson AH, et al. Effects of artificial gravity during bed rest on bone metabolism in humans. *J Appl Physiol.* 2009;107:47-53.
679. Eyre DR, Dickson IR, Van Ness K. Collagen cross-linking in human bone and articular cartilage. *Biochem J.* 1988;252:405-500.
680. Uebelhart D, Gineyts E, Chapuy MC, Delmas PD. Urinary excretion of pyridinium crosslinks: a new marker of bone resorption in metabolic bone disease. *Bone Miner.* 1990;8:87-96.
681. Robins SP, Woitge H, Hesley R, Ju J, Seyedin S, Seibel MJ. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. *J Bone Miner Res.* 1994;9:1643-9.
682. Seyedin SM, Kung VT, Daniloff YN, Hesley RP, Gomez B, Nielsen LA, et al. Immunoassay for urinary pyridinoline: the new marker of bone resorption. *J Bone Miner Res.* 1993;8:635-41.
683. Hawkey A. The importance of exercising in space. *Interdiscip Sci Rev.* 2003;28:130-8.
684. Convertino VA. Planning strategies for development of effective exercise and nutrition countermeasures for long-duration space flight. *Nutrition.* 2002;18:880-8.
685. Oganov VS, Bogomolov VV. [Human bone system in microgravity: review of research data, hypotheses and predictability of musculoskeletal system state in extended (exploration) missions]. *Aviakosm Ekolog Med.* 2009;43:3-12.
686. Cavanagh PR, Licata AA, Rice AJ. Exercise and pharmacological countermeasures for bone loss during long-duration space flight. *Gravit Space Biol Bull.* 2005;18:39-58.
687. Novotny SC, Perusek GP, Rice AJ, Comstock BA, Bansal A, Cavanagh PR. A harness for enhanced comfort and loading during treadmill exercise in space. *Acta Astronaut.* 2013;89:205-14.
688. Rittweger J, Frost HM, Schiessl H, Ohshima H, Alkner B, Tesch P, et al. Muscle atrophy and bone loss after 90 days' bed rest and the effects of flywheel resistive exercise and pamidronate: results from the LTBR study. *Bone.* 2005;36:1019-29.
689. Watanabe Y, Ohshima H, Mizuno K, Sekiguchi C, Fukunaga M, Kohri K, et al. Intravenous pamidronate prevents femoral bone loss and renal stone formation during 90-day bed rest. *J Bone Miner Res.* 2004;19:1771-8.
690. Grigoriev AI, Morukov BV, Oganov VS, Rakhmanov AS, Buravkova LB. Effect of exercise and bisphosphonate on mineral balance and bone density during 360 day antiorthostatic hypokinesia. *J Bone Miner Res.* 1992;7 Suppl 2:S449-55.
691. Zwart SR, Hargens AR, Lee SM, Macias BR, Watenpaugh DE, Tse K, et al. Lower body negative pressure treadmill exercise as a countermeasure for bed rest-induced bone loss in female identical twins. *Bone.* 2007;40:529-37.
692. Thomsen JS, Morukov BV, Vico L, Alexandre C, Saporin PI, Gowin W. Cancellous bone structure of iliac crest biopsies following 370 days of head-down bed rest. *Aviat Space Environ Med.* 2005;76:915-22.
693. Berg HE, Eiken O, Miklavcic L, Mekjavic IB. Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. *Eur J Appl Physiol.* 2007;99:283-9.
694. Young LR, Paloski WH. Short radius intermittent centrifugation as a countermeasure to bed-rest and 0-G deconditioning: IMAG pilot study summary and recommendations for research. *J Gravit Physiol.* 2007;14:P31-3.
695. Stenger MB, Evans JM, Knapp CF, Lee SM, Phillips TR, Perez SA, et al. Artificial gravity training reduces bed rest-induced cardiovascular deconditioning. *Eur J Appl Physiol.* 2012;112:605-16.
696. Symons TB, Sheffield-Moore M, Chinkes DL, Ferrando AA, Paddon-Jones D. Artificial gravity maintains skeletal muscle protein synthesis during 21 days of simulated microgravity. *J Appl Physiol.* 2009;107:34-8.
697. Smith SM, Castaneda-Sceppa C, O'Brien KO, Abrams SA, Gillman P, Brooks NE, et al. Calcium kinetics during bed rest with artificial gravity and exercise countermeasures. *Osteoporos Int.* 2014;25:2237-44.
698. Yang Y, Kaplan A, Pierre M, Adams G, Cavanagh P, Takahashi C, et al. Space cycle: a human-powered centrifuge that can be used for hypergravity resistance training. *Aviat Space Environ Med.* 2007;78:2-9.

699. Vernikos J. Artificial gravity intermittent centrifugation as a space flight countermeasure. *J Gravit Physiol.* 1997;4:P13-6.
700. Clément G, Buckley AP, editors. Artificial gravity. New York: Springer; 2007.
701. Vernikos J, Ludwig DA, Ertl AC, Wade CE, Keil L, O'Hara D. Effect of standing or walking on physiological changes induced by head down bed rest: implications for spaceflight. *Aviat Space Environ Med.* 1996;67:1069-79.
702. Greenleaf JE, Chou JL, Stad NJ, Leftheriotis GP, Arndt NF, Jackson CG, et al. Short-arm (1.9 m) +2.2 Gz acceleration: isotonic exercise load-O<sub>2</sub> uptake relationship. *Aviat Space Environ Med.* 1999;70:1173-82.
703. Yang Y, Baker M, Graf S, Larson J, Caiozzo VJ. Hypergravity resistance exercise: the use of artificial gravity as potential countermeasure to microgravity. *J Appl Physiol.* 2007;103:1879-87.
704. Naumann FL, Bennell KL, Wark JD. The effects of +Gz force on the bone mineral density of fighter pilots. *Aviat Space Environ Med.* 2001;72:177-81.
705. Naumann FL, Grant MC, Dhaliwal SS. Changes in cervical spine bone mineral density in response to flight training. *Aviat Space Environ Med.* 2004;75:255-9.
706. Iwase S, Takada H, Watanabe Y, Ishida K, Akima H, Katayama K, et al. Effect of centrifuge-induced artificial gravity and ergometric exercise on cardiovascular deconditioning, myatrophy, and osteoporosis induced by a -6 degrees head-down bedrest. *J Gravit Physiol.* 2004;11:P243-4.
707. Clement G, Pavy-Le Traon A. Centrifugation as a countermeasure during actual and simulated microgravity: a review. *Eur J Appl Physiol.* 2004;92:235-48.
708. Heer M, Baecker N, Zwart SR, Smith SM. Interactions between artificial gravity, affected physiological systems, and nutrition. In: Clement G, Buckley A, editors. Artificial gravity. New York: Springer; 2007. p. 249-70.
709. Holguin N, Uzer G, Chiang FP, Rubin C, Judex S. Brief daily exposure to low-intensity vibration mitigates the degradation of the intervertebral disc in a frequency-specific manner. *J Appl Physiol.* 2011;111:1846-53.
710. Kiel DP, Hannan MT, Barton BA, Bouxsein ML, Lang TF, Brown KM, et al. Insights from the conduct of a device trial in older persons: low magnitude mechanical stimulation for musculoskeletal health. *Clin Trials.* 2010;7:354-67.
711. Xie L, Rubin C, Judex S. Enhancement of the adolescent murine musculoskeletal system using low-level mechanical vibrations. *J Appl Physiol.* 2008;104:1056-62.
712. Holguin N, Muir J, Rubin C, Judex S. Short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. *Spine J.* 2009;9:470-7.
713. Belavy DL, Beller G, Armbrecht G, Perschel FH, Fitzner R, Bock O, et al. Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bed rest. *Osteoporos Int.* 2011;22:1581-91.
714. Belavy DL, Hides JA, Wilson SJ, Stanton W, Dimeo FC, Rittweger J, et al. Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bed-rest. *Spine.* 2008;33:E121-31.
715. Rittweger J, Beller G, Armbrecht G, Mulder E, Buehring B, Gast U, et al. Prevention of bone loss during 56 days of strict bed rest by side-alternating resistive vibration exercise. *Bone.* 2010;46:137-47.
716. Wang H, Wan Y, Tam KF, Ling S, Bai Y, Deng Y, et al. Resistive vibration exercise retards bone loss in weight-bearing skeletons during 60 days bed rest. *Osteoporos Int.* 2012;23:2169-78.
717. Lockwood DR, Vogel JM, Schneider VS, Hulley SB. Effect of the diphosphonate EHDP on bone mineral metabolism during prolonged bed rest. *J Clin Endocrinol Metab.* 1975;41:533-41.
718. Iwamoto J, Takeda T, Sato Y. Interventions to prevent bone loss in astronauts during space flight. *Keio J Med.* 2005;54:55-9.
719. LeBlanc AD, Driscoll TB, Shackelford LC, Evans HJ, Rianon NJ, Smith SM, et al. Alendronate as an effective countermeasure to disuse induced bone loss. *J Musculoskelet Neuronal Interact.* 2002;2:335-43.
720. Shapiro J, Smith B, Beck T, Ballard P, Dapthary M, Brintzenhofesoc K, et al. Treatment with zoledronic acid ameliorates negative geometric changes in the proximal femur following acute spinal cord injury. *Calcif Tissue Int.* 2007;80:316-22.
721. Minaire P, Berard E, Meunier PJ, Edouard C, Goedert G, Pilonchery G. Effects of disodium dichloromethylene diphosphonate on bone loss in paraplegic patients. *J Clin Invest.* 1981;68:1086-92.
722. Maheshwari UR, Leybin L, McDonald JT, Schneider VS, Newbrun E, Hodge HC. Effect of dichloromethylene diphosphonate on fluoride balance in healthy men. *J Dent Res.* 1983;62:559-61.

723. Okada A, Ohshima H, Itoh Y, Yasui T, Tozawa K, Kohri K. Risk of renal stone formation induced by long-term bed rest could be decreased by premedication with bisphosphonate and increased by resistive exercise. *Int J Urol*. 2008;15:630-5.
724. Chappard D, Alexandre C, Palle S, Vico L, Morukov BV, Rodionova SS, et al. Effects of a bisphosphonate (1-hydroxy ethylidene-1,1 bisphosphonic acid) on osteoclast number during prolonged bed rest in healthy humans. *Metabolism*. 1989;38:822-5.
725. Li CY, Majeska RJ, Laudier DM, Mann R, Schaffler MB. High-dose risedronate treatment partially preserves cancellous bone mass and microarchitecture during long-term disuse. *Bone*. 2005;37:287-95.
726. Li CY, Price C, Delisser K, Nasser P, Laudier D, Clement M, et al. Long-term disuse osteoporosis seems less sensitive to bisphosphonate treatment than other osteoporosis. *J Bone Miner Res*. 2005;20:117-24.
727. Wimalawansa SM, Wimalawansa SJ. Simulated weightlessness-induced attenuation of testosterone production may be responsible for bone loss. *Endocrine*. 1999;10:253-60.
728. Strollo F, Boitani C, Basciani S, Pecorelli L, Palumbo D, Borgia L, et al. The pituitary-testicular axis in microgravity: analogies with the aging male syndrome. *J Endocrinol Invest*. 2005;28:78-83.
729. Strollo F. Hormonal changes in humans during spaceflight. *Adv Space Biol Med*. 1999;7:99-129.
730. Strollo F, Barger L, Fuller C. Testosterone urinary excretion rate increases during hypergravity in male monkeys. *J Gravit Physiol*. 2000;7:P181-2.
731. Strollo F, Masini MA, Pastorino M, Ricci F, Vadrucci S, Cogoli-Greuter M, et al. Microgravity-induced alterations in cultured testicular cells. *J Gravit Physiol*. 2004;11:P187-8.
732. Strollo F, Riondino G, Harris B, Strollo G, Casarosa E, Mangrossa N, et al. The effect of microgravity on testicular androgen secretion. *Aviat Space Environ Med*. 1998;69:133-6.
733. Strollo F, Strollo G, More M, Ferretti C, Mangrossa N, Casarosa E, et al. Changes in human adrenal and gonadal function onboard Spacelab. *J Gravit Physiol*. 1997;4:P103-4.
734. Strollo F, Strollo G, Morè M, Mangrossa N, Riondino G, Luisi M, et al. Space flight induces endocrine changes at both the pituitary and peripheral level in the absence of any major chronobiologic disturbances. In: Sahn PR, Keller MH, Schiewe B, editors. *Proceedings of the Norderney Symposium on Scientific Results of the German Spacelab Mission D-2*. Norderney, Germany: Wissenschaftliche Projectführung D-2; 1995. p. 743-7.
735. Hulley SB, Vogel JM, Donaldson CL, Bayers JH, Friedman RJ, Rosen SN. The effect of supplemental oral phosphate on the bone mineral changes during prolonged bed rest. *J Clin Invest*. 1971;50:2506-18.
736. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. *J Bone Miner Res*. 2003;18:1206-16.
737. Nile SH, Park SW. Edible berries: Bioactive components and their effect on human health. *Nutrition*. 2014;30:134-44.
738. Chen YM, Ho SC, Woo JL. Greater fruit and vegetable intake is associated with increased bone mass among postmenopausal Chinese women. *Br J Nutr*. 2006;96:745-51.
739. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, et al. Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. *Am J Clin Nutr*. 2006;83:1420-8.
740. Rivas A, Romero A, Mariscal-Arcas M, Monteagudo C, Feriche B, Lorenzo ML, et al. Mediterranean diet and bone mineral density in two age groups of women. *Int J Food Sci Nutr*. 2013;64:155-61.
741. Byberg L, Bellavia A, Orsini N, Wolk A, Michaelsson K. Fruit and vegetable intake and risk of hip fracture: A cohort study of Swedish men and women. *J Bone Miner Res*. doi: 10.1002/jbmr.2384. [Epub ahead of print]. 2014 Oct 7.
742. Xie HL, Wu BH, Xue WQ, He MG, Fan F, Ouyang WF, et al. Greater intake of fruit and vegetables is associated with a lower risk of osteoporotic hip fractures in elderly Chinese: a 1:1 matched case-control study. *Osteoporos Int*. 2013;24:2827-36.
743. Dai Z, Butler LM, van Dam RM, Ang LW, Yuan JM, Koh WP. Adherence to a vegetable-fruit-soy dietary pattern or the Alternative Healthy Eating Index is associated with lower hip fracture risk among Singapore Chinese. *J Nutr*. 2014;144:511-8.
744. Zeng FF, Wu BH, Fan F, Xie HL, Xue WQ, Zhu HL, et al. Dietary patterns and the risk of hip fractures in elderly Chinese: a matched case-control study. *J Clin Endocrinol Metab*. 2013;98:2347-55.
745. Hardcastle AC, Aucott L, Fraser WD, Reid DM, Macdonald HM. Dietary patterns, bone resorption and bone mineral density in early post-menopausal Scottish women. *Eur J Clin Nutr*. 2011;65:378-85.
746. Lanham-New SA. Fruit and vegetables: the unexpected natural answer to the question of osteoporosis prevention? *Am J Clin Nutr*. 2006;83:1254-5.

## *Risk Factor of Inadequate Nutrition*

747. Sacco SM, Horcajada MN, Offord E. Phytonutrients for bone health during ageing. *Br J Clin Pharmacol*. 2013;75:697-707.
748. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: vegetables and fruit in the prevention of chronic diseases. *Eur J Nutr*. 2012;51:637-63.
749. Oyebo O, Gordon-Dseagu V, Walker A, Mindell JS. Fruit and vegetable consumption and all-cause, cancer and CVD mortality: analysis of Health Survey for England data. *J Epidemiol Community Health*. 2014;68:856-62.
750. Macready AL, George TW, Chong MF, Alimbetov DS, Jin Y, Vidal A, et al. Flavonoid-rich fruit and vegetables improve microvascular reactivity and inflammatory status in men at risk of cardiovascular disease--FLAVURS: a randomized controlled trial. *Am J Clin Nutr*. 2014;99:479-89.
751. Dai Z, Wang R, Ang LW, Low YL, Yuan JM, Koh WP. Protective effects of dietary carotenoids on risk of hip fracture in men: the singapore chinese health study. *J Bone Miner Res*. 2014;29:408-17.
752. Mackinnon ES, Rao AV, Josse RG, Rao LG. Supplementation with the antioxidant lycopene significantly decreases oxidative stress parameters and the bone resorption marker N-telopeptide of type I collagen in postmenopausal women. *Osteoporos Int*. 2011;22:1091-101.
753. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. *J Bone Miner Res*. 2009;24:1086-94.
754. Mackinnon ES, Rao AV, Rao LG. Dietary restriction of lycopene for a period of one month resulted in significantly increased biomarkers of oxidative stress and bone resorption in postmenopausal women. *J Nutr Health Aging*. 2011;15:133-8.
755. Schneider VS, LeBlanc A, Huntoon CL. Prevention of space flight induced soft tissue calcification and disuse osteoporosis. *Acta Astronaut*. 1993;29:139-40.
756. Parfitt AM. Bone effects of space flight: analysis by quantum concept of bone remodelling. *Acta Astronaut*. 1981;8:1083-90.
757. Giangregorio L, Blimkie CJ. Skeletal adaptations to alterations in weight-bearing activity: a comparison of models of disuse osteoporosis. *Sports Med*. 2002;32:459-76.
758. Scott JM, Warburton DE, Williams D, Whelan S, Krassioukov A. Challenges, concerns and common problems: physiological consequences of spinal cord injury and microgravity. *Spinal Cord*. 2011;49:4-16.
759. Wolf J, Vermeer C. Potential effect of vitamin K on microgravity-induced bone loss. *J Gravit Physiol*. 1996;3:29-32.
760. Anderson JJB, Klemmer PJ, Watts MLS, Garner SC, Calvo MS. Phosphorus. In: Bowman BA, Russell RM, editors. *Present knowledge in nutrition*. 9th ed. Washington, DC: International Life Sciences Institute Press; 2006. p. 383-99.
761. Calvo MS, Moshfegh AJ, Tucker KL. Assessing the health impact of phosphorus in the food supply: issues and considerations. *Adv Nutr*. 2014;5:104-13.
762. Chang AR, Lazo M, Appel LJ, Gutierrez OM, Grams ME. High dietary phosphorus intake is associated with all-cause mortality: results from NHANES III. *Am J Clin Nutr*. 2014;99:320-7.
763. Takeda E, Yamamoto H, Yamanaka-Okumura H, Taketani Y. Increasing dietary phosphorus intake from food additives: potential for negative impact on bone health. *Adv Nutr*. 2014;5:92-7.
764. Schuille PO, Schmiedl A, Herrmann U, Fan J, Gottlieb D, Manoharan M, et al. Magnesium, citrate, magnesium citrate and magnesium-alkali citrate as modulators of calcium oxalate crystallization in urine: observations in patients with recurrent idiopathic calcium urolithiasis. *Urol Res*. 1999;27:117-26.
765. Whitson PA, Pietrzyk RA, Jones JA, Nelman-Gonzalez M, Hudson EK, Sams CF. Effect of potassium citrate therapy on the risk of renal stone formation during spaceflight. *J Urol*. 2009;182:2490-6.
766. Holt RA, Urui-Adams JY, Keen CL. Zinc. In: Erdman JW, Jr., MacDonald IA, Zeisel SH, editors. *Present knowledge in nutrition*. 9th ed. Washington, DC: International Life Sciences Institute Press; 2010. p. 521-39.
767. Garcia HD, Hays SM, Tsuji JS. Modeling of blood lead levels in astronauts exposed to lead from microgravity-accelerated bone loss. *Aviat Space Environ Med*. 2013;84:1229-34.
768. Dawson-Hughes B. Interaction of dietary calcium and protein in bone health in humans. *J Nutr*. 2003;133:852S-4S.
769. Rafferty K, Heaney RP. Nutrient effects on the calcium economy: emphasizing the potassium controversy. *J Nutr*. 2008;138:166S-71S.
770. Dawson-Hughes B. Calcium and protein in bone health. *Proc Nutr Soc*. 2003;62:505-9.

771. Marckmann P, Osther P, Pedersen AN, Jespersen B. High-protein diets and renal health. *J Ren Nutr.* 2014. doi: 10.1053/j.jrn.2014.06.002. [Epub ahead of print].
772. Hegsted M, Linkswiler HM. Long-term effects of level of protein intake on calcium metabolism in young adult women. *J Nutr.* 1981;111:244-51.
773. Borghi L, Schianchi T, Meschi T, Guerra A, Allegri F, Maggiore U, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med.* 2002;346:77-84.
774. Zwart SR, Hargens AR, Smith SM. The ratio of animal protein intake to potassium intake is a predictor of bone resorption in space flight analogues and in ambulatory subjects. *Am J Clin Nutr.* 2004;80:1058-65.
775. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J Clin Endocrinol Metab.* 2005;90:26-31.
776. Breslau NA, Brinkley L, Hill KD, Pak CY. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endocrinol Metab.* 1988;66:140-6.
777. Kaneko K, Masaki U, Aikyo M, Yabuki K, Haga A, Matoba C, et al. Urinary calcium and calcium balance in young women affected by high protein diet of soy protein isolate and adding sulfur-containing amino acids and/or potassium. *J Nutr Sci Vitaminol (Tokyo).* 1990;36:105-16.
778. Zwart SR, Davis-Street JE, Paddon-Jones D, Ferrando AA, Wolfe RR, Smith SM. Amino acid supplementation alters bone metabolism during simulated weightlessness. *J Appl Physiol.* 2005;99:134-40.
779. Fenton TR, Eliasziw M, Lyon AW, Tough SC, Hanley DA. Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis. *Am J Clin Nutr.* 2008;88:1159-66.
780. Morgan JL, Skulan JL, Gordon GW, Romaniello SJ, Smith SM, Anbar AD. Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. *Proc Natl Acad Sci U S A.* 2012;109:9989-94.
781. Russell WA, Papanastassiou DA, Tombrello TA. Ca isotope fractionation on earth and other solar-system materials. *Geochim Cosmochim Acta.* 1978;42:1075-90.
782. Morgan JL, Gordon GW, Arrua RC, Skulan JL, Anbar AD, Bullen TD. High-precision measurement of variations in calcium isotope ratios in urine by multiple collector inductively coupled plasma mass spectrometry. *Anal Chem.* 2011;83:6956-62.
783. Skulan J, Bullen T, Anbar AD, Puzas JE, Shackelford L, LeBlanc A, et al. Natural calcium isotopic composition of urine as a marker of bone mineral balance. *Clin Chem.* 2007;53:1155-8.
784. Skulan JL, DePaolo DJ. Calcium isotope fractionation between soft and mineralized tissue as a monitor of calcium use in vertebrates. *Proc Natl Acad Sci U S A.* 1999;96:13709-13.
785. Fitts RH, Riley DR, Widrick JJ. Invited review: microgravity and skeletal muscle. *J Appl Physiol.* 2000;89:823-39.
786. Adams GR, Caiozzo VJ, Baldwin KM. Skeletal muscle unweighting: spaceflight and ground-based models. *J Appl Physiol.* 2003;95:2185-201.
787. LeBlanc A, Rowe R, Schneider V, Evans H, Hedrick T. Regional muscle loss after short duration spaceflight. *Aviat Space Environ Med.* 1995;66:1151-4.
788. Desplanches D. Structural and functional adaptations of skeletal muscle to weightlessness. *Int J Sports Med.* 1997;18 Suppl 4:S259-64.
789. Grogor'eva LS, Kozlovskaia IB. [Effect of weightlessness and hypokinesia on the velocity-strength properties of human muscles]. *Kosm Biol Aviakosm Med.* 1987;21:27-30.
790. Tesch PA, Berg HE, Bring D, Evans HJ, LeBlanc AD. Effects of 17-day spaceflight on knee extensor muscle function and size. *Eur J Appl Physiol.* 2005;93:463-8.
791. Gopalakrishnan R, Genc KO, Rice AJ, Lee SM, Evans HJ, Maender CC, et al. Muscle volume, strength, endurance, and exercise loads during 6-month missions in space. *Aviat Space Environ Med.* 2010;81:91-102.
792. Michel EL, Rummel JA, Sawin CF, Buderer MC, Lem JD. Results of Skylab medical experiment M-171 - Metabolic Activity. In: Johnston RS, Dietlein LF, editors. *Biomedical results from Skylab (NASA SP-377)*. Washington, DC: National Aeronautics and Space Administration; 1977. p. 372-87.
793. Antonutto G, Capelli C, Girardis M, Zamparo P, di Prampero PE. Effects of microgravity on maximal power of lower limbs during very short efforts in humans. *J Appl Physiol.* 1999;86:85-92.
794. Zange J, Muller K, Schuber M, Wackerhage H, Hoffmann U, Gunther RW, et al. Changes in calf muscle performance, energy metabolism, and muscle volume caused by long-term stay on space station MIR. *Int J Sports Med.* 1997;18 Suppl 4:S308-9.

795. Bajotto G, Shimomura Y. Determinants of disuse-induced skeletal muscle atrophy: exercise and nutrition countermeasures to prevent protein loss. *J Nutr Sci Vitaminol (Tokyo)*. 2006;52:233-47.
796. Day MK, Allen DL, Mohajerani L, Greenisen MC, Roy RR, Edgerton VR. Adaptations of human skeletal muscle fibers to spaceflight. *J Gravit Physiol*. 1995;2:P47-50.
797. Narici MV, de Boer MD. Disuse of the musculo-skeletal system in space and on earth. *Eur J Appl Physiol*. 2011;111:403-20.
798. Convertino VA. Physiological adaptations to weightlessness: effects on exercise and work performance. *Exerc Sport Sci Rev*. 1990;18:119-66.
799. Baldwin KM. Effect of spaceflight on the functional, biochemical, and metabolic properties of skeletal muscle. *Med Sci Sports Exerc*. 1996;28:983-7.
800. Bodine SC. Disuse-induced muscle wasting. *Int J Biochem Cell Biol*. 2013;45:2200-8.
801. Stein TP. Nutrition and muscle loss in humans during spaceflight. *Adv Space Biol Med*. 1999;7:49-97.
802. Smith GI, Patterson BW, Mittendorfer B. Human muscle protein turnover--why is it so variable? *J Appl Physiol*. 2011;110:480-91.
803. Stein TP, Leskiw MJ, Schluter MD. Effect of spaceflight on human protein metabolism. *Am J Physiol Endocrinol Metab*. 1993;264:E824-8.
804. Stein TP. Protein and muscle homeostasis: the role of nutrition. In: Lane HW, Schoeller DA, editors. *Nutrition in spaceflight and weightlessness models*. Boca Raton, FL: CRC Press; 2000. p. 141-77.
805. Ushakov AS, Vlasova TF. Free amino acids in human blood plasma during space flights. *Aviat Space Environ Med*. 1976;47:1061-4.
806. Ushakov AS, Vlasova TF. Amino acid spectrum of human blood plasma during space flight and in antiorthostatic hypokinesia. *Life Sci Space Res*. 1976;14:257-62.
807. Stein TP, Schluter MD. Plasma amino acids during human spaceflight. *Aviat Space Environ Med*. 1999;70:250-5.
808. Navasiolava NM, Custaud MA, Tomilovskaya ES, Larina IM, Mano T, Gauquelin-Koch G, et al. Long-term dry immersion: review and prospects. *Eur J Appl Physiol*. 2011;111:1235-60.
809. Biolo G, Ciocchi B, Lebenstedt M, Barazzoni R, Zanetti M, Platen P, et al. Short-term bed rest impairs amino acid-induced protein anabolism in humans. *J Physiol*. 2004;558:381-8.
810. Biolo G, Ciocchi B, Lebenstedt M, Heer M, Guarnieri G. Sensitivity of whole body protein synthesis to amino acid administration during short-term bed rest. *J Gravit Physiol*. 2002;9:P197-8.
811. Drummond MJ, Dickinson JM, Fry CS, Walker DK, Gundermann DM, Reidy PT, et al. Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. *Am J Physiol Endocrinol Metab*. 2012;302:E1113-22.
812. Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol Endocrinol Metab*. 1996;270:E627-E33.
813. Ferrando AA, Tipton KD, Bamman MM, Wolfe RR. Resistance exercise maintains skeletal muscle protein synthesis during bed rest. *J Appl Physiol (1985)*. 1997;82:807-10.
814. Stuart CA, Shangraw RE, Peters EJ, Wolfe RR. Effect of dietary protein on bed-rest-related changes in whole-body-protein synthesis. *Am J Clin Nutr*. 1990;52:509-14.
815. Glover EI, Phillips SM, Oates BR, Tang JE, Tarnopolsky MA, Selby A, et al. Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *J Physiol*. 2008;586:6049-61.
816. Blanc S, Normand S, Ritz P, Pachiardi C, Vico L, Gharib C, et al. Energy and water metabolism, body composition, and hormonal changes induced by 42 days of enforced inactivity and simulated weightlessness. *J Clin Endocrinol Metab*. 1998;83:4289-97.
817. Rambaut PC, Leach CS, Whedon GD. A study of metabolic balance in crewmembers of Skylab IV. *Acta Astronaut*. 1979;6:1313-22.
818. Heer M, De Santo NG, Cirillo M, Drummer C. Body mass changes, energy, and protein metabolism in space. *Am J Kidney Dis*. 2001;38:691-5.
819. Biolo G, Agostini F, Simunic B, Sturma M, Torelli L, Preiser JC, et al. Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest. *Am J Clin Nutr*. 2008;88:950-8.
820. Drummond MJ, Timmerman KL, Markofski MM, Walker DK, Dickinson JM, Jamaluddin M, et al. Short-term bed rest increases TLR4 and IL-6 expression in skeletal muscle of older adults. *Am J Physiol Regul Integr Comp Physiol*. 2013;305:R216-23.

## *Risk Factor of Inadequate Nutrition*

821. Bosutti A, Malaponte G, Zanetti M, Castellino P, Heer M, Guarnieri G, et al. Calorie restriction modulates inactivity-induced changes in the inflammatory markers C-reactive protein and pentraxin-3. *J Clin Endocrinol Metab.* 2008;93:3226-9.
822. Millet C, Custaud MA, Mailliet A, Allevard AM, Duvareille M, Gauquelin-Koch G, et al. Endocrine responses to 7 days of head-down bed rest and orthostatic tests in men and women. *Clin Physiol.* 2001;21:172-83.
823. Paddon-Jones D, Sheffield-Moore M, Cree MG, Hewlings SJ, Aarsland A, Wolfe RR, et al. Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. *J Clin Endocrinol Metab.* 2006;91:4836-41.
824. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Aarsland A, Wolfe RR, Ferrando AA. The catabolic effects of prolonged inactivity and acute hypercortisolemia are offset by dietary supplementation. *J Clin Endocrinol Metab.* 2005;90:1453-9.
825. Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. *Am J Physiol Cell Physiol.* 2007;293:C313-20.
826. Baldwin KM. Future research directions in seeking countermeasures to weightlessness. *J Gravit Physiol.* 1995;2:P51-3.
827. di Prampero PE, Narici MV. Muscles in microgravity: from fibres to human motion. *J Biomech.* 2003;36:403-12.
828. Trappe S, Costill D, Gallagher P, Creer A, Peters JR, Evans H, et al. Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. *J Appl Physiol.* 2009;106:1159-68.
829. LeBlanc A, Lin C, Rowe R, Belichenko O, Sinitsyn V, Shenkman B, et al. Muscle loss after long duration spaceflight on Mir 18/STS-71 [abstract]. *AIAA Life Sciences and Space Medicine Conference 1996.* p. 53-4, Abstract 96-LS-71.
830. Tesch PA, Ekberg A, Lindquist DM, Trieschmann JT. Muscle hypertrophy following 5-week resistance training using a non-gravity-dependent exercise system. *Acta Physiol Scand.* 2004;180:89-98.
831. Alkner BA, Berg HE, Kozlovskaya I, Sayenko D, Tesch PA. Effects of strength training, using a gravity-independent exercise system, performed during 110 days of simulated space station confinement. *Eur J Appl Physiol.* 2003;90:44-9.
832. Alkner BA, Tesch PA. Knee extensor and plantar flexor muscle size and function following 90 days of bed rest with or without resistance exercise. *Eur J Appl Physiol.* 2004;93:294-305.
833. Reeves ND, Maganaris CN, Ferretti G, Narici MV. Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. *J Appl Physiol.* 2005;98:2278-86.
834. Rittweger J, Felsenberg D, Maganaris C, Ferretti JL. Vertical jump performance after 90 days bed rest with and without flywheel resistive exercise, including a 180 days follow-up. *Eur J Appl Physiol.* 2007;100:427-36.
835. Milesi S, Capelli C, Denoth J, Hutchinson T, di Prampero PE, Stussi E. Effects of 17 days bed rest on the maximal isometric torque of the flexors and extensors of the ankle. *J Gravit Physiol.* 1997;4:P125-6.
836. Schwandt DF, Whalen RT, Watenpaugh DE, Parazyński SE, Hargens AR. Development of exercise devices to minimize musculoskeletal and cardiovascular deconditioning in microgravity. *Physiologist.* 1991;34 Suppl 1:S189-90.
837. Greenleaf JE, Bulbulian R, Bernauer EM, Haskell WL, Moore T. Exercise-training protocols for astronauts in microgravity. *J Appl Physiol.* 1989;67:2191-204.
838. Macias BR, Groppo ER, Eastlack RK, Watenpaugh DE, Lee SM, Schneider SM, et al. Space exercise and Earth benefits. *Curr Pharm Biotechnol.* 2005;6:305-17.
839. Krainski F, Hastings JL, Heinicke K, Romain N, Pacini EL, Snell PG, et al. The effect of rowing ergometry and resistive exercise on skeletal muscle structure and function during bed rest. *J Appl Physiol (1985).* 2014;116:1569-81.
840. Ploutz-Snyder LL, Downs M, Ryder J, Hackney K, Scott J, Buxton R, et al. Integrated resistance and aerobic exercise protects fitness during bed rest. *Med Sci Sports Exerc.* 2014;46:358-68.
841. Rubin C, Recker R, Cullen D, Ryaby J, McCabe J, McLeod K. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. *J Bone Miner Res.* 2004;19:343-51.
842. Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P, et al. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. *J Appl Physiol.* 2005;99:1293-300.

843. Zange J, Mester J, Heer M, Kluge G, Liphardt AM. 20-Hz whole body vibration training fails to counteract the decrease in leg muscle volume caused by 14 days of 6 degrees head down tilt bed rest. *Eur J Appl Physiol.* 2009;105:271-7.
844. Belavy DL, Armbrecht G, Gast U, Richardson CA, Hides JA, Felsenberg D. Countermeasures against lumbar spine deconditioning in prolonged bed rest: resistive exercise with and without whole body vibration. *J Appl Physiol.* 2010;109:1801-11.
845. Mikhael M, Orr R, Fiatarone Singh MA. The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: a systematic review of the literature. *Maturitas.* 2010;66:150-7.
846. Prisby RD, Lafage-Proust MH, Malaval L, Belli A, Vico L. Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. *Ageing Res Rev.* 2008;7:319-29.
847. Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, et al. Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bed-rest. *Int J Sports Med.* 2006;27:553-9.
848. Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat Rec.* 1987;219:1-9.
849. Frost HM. Bone's mechanostat: a 2003 update. *Anat Rec A Discov Mol Cell Evol Biol.* 2003;275:1081-101.
850. Baecker N, Frings-Meuthen P, Heer M, Mester J, Liphardt AM. Effects of vibration training on bone metabolism: results from a short-term bed rest study. *Eur J Appl Physiol.* 2012;112:1741-50.
851. Armbrecht G, Belavy DL, Gast U, Bongrazio M, Touby F, Beller G, et al. Resistive vibration exercise attenuates bone and muscle atrophy in 56 days of bed rest: biochemical markers of bone metabolism. *Osteoporos Int.* 2010;21:597-607.
852. Belavy DL, Beller G, Ritter Z, Felsenberg D. Bone structure and density via HR-pQCT in 60d bed-rest, 2-years recovery with and without countermeasures. *J Musculoskelet Neuronal Interact.* 2011;11:215-26.
853. Belavy DL, Miokovic T, Armbrecht G, Rittweger J, Felsenberg D. Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest. *J Musculoskelet Neuronal Interact.* 2009;9:225-35.
854. Trudel G, Coletta E, Cameron I, Belavy DL, Lecompte M, Armbrecht G, et al. Resistive exercises, with or without whole body vibration, prevent vertebral marrow fat accumulation during 60 days of head-down tilt bed rest in men. *J Appl Physiol.* 2012;112:1824-31.
855. Strollo F, Strollo G, More M, Bollanti L, Ciarmatori A, Longo E, et al. Hormonal adaptation to real and simulated microgravity. *J Gravitational Physiol.* 1998;5:P89-P92.
856. Wade CE, Stanford KI, Stein TP, Greenleaf JE. Intensive exercise training suppresses testosterone during bed rest. *J Appl Physiol.* 2005;99:59-63.
857. Space Science Board. *Endocrinology. Life beyond the Earth's environment: the biology of living organisms in space.* Washington, D.C.: National Academy of Sciences; 1979. p. 65.
858. Strollo F, Norsk P, Roecker L, Strollo G, More M, Bollanti L, et al. Indirect evidence of CNS adrenergic pathways activation during spaceflight. *Aviat Space Environ Med.* 1998;69:777-80.
859. Alemany JA, Nindl BC, Kellogg MD, Tharion WJ, Young AJ, Montain SJ. Effects of dietary protein content on IGF-I, testosterone, and body composition during 8 days of severe energy deficit and arduous physical activity. *J Appl Physiol.* 2008;105:58-64.
860. Kyrolainen H, Karinkanta J, Santtila M, Koski H, Mantysaari M, Pullinen T. Hormonal responses during a prolonged military field exercise with variable exercise intensity. *Eur J Appl Physiol.* 2008;102:539-46.
861. Cangemi R, Friedmann AJ, Holloszy JO, Fontana L. Long-term effects of calorie restriction on serum sex-hormone concentrations in men. *Aging Cell.* 2010;9:236-42.
862. Murdaca G, Setti M, Brenci S, Fenoglio D, Lantieri P, Indiveri F, et al. Modifications of immunological and neuro-endocrine parameters induced by antiorthostatic bed-rest in human healthy volunteers. *Minerva Med.* 2003;94:363-78.
863. Greenleaf JE, Bernauer EM, Ertl AC, Trowbridge TS, Wade CE. Work capacity during 30 days of bed rest with isotonic and isokinetic exercise training. *J Appl Physiol.* 1989;67:1820-6.
864. Zorbas YG, Naexu KA, Federenko YF. Blood serum biochemical changes in physically conditioned and unconditioned subjects during bed rest and chronic hyperhydration. *Clin Exp Pharmacol Physiol.* 1992;19:137-45.
865. Smorawinski J, Nazar K, Kaciuba-Uscilko H, Kaminska E, Cybulski G, Kodrzycka A, et al. Effects of 3-day bed rest on physiological responses to graded exercise in athletes and sedentary men. *J Appl Physiol.* 2001;91:249-57.
866. Tou J, Ronca A, Grindeland R, Wade C. Models to study gravitational biology of Mammalian reproduction. *Biol Reprod.* 2002;67:1681-7.

867. Vasques M, Lang C, Grindeland RE, Roy RR, Daunton N, Bigbee AJ, et al. Comparison of hyper- and microgravity on rat muscle, organ weights and selected plasma constituents. *Aviat Space Environ Med.* 1998;69:A2-8.
868. Ortiz RM, Wade CE, Morey-Holton E. Urinary excretion of LH and testosterone from male rats during exposure to increased gravity: post-spaceflight and centrifugation. *Proc Soc Exp Biol Med.* 2000;225:98-102.
869. Amann RP, Deaver DR, Zirkin BR, Grills GS, Sapp WJ, Veeramachaneni DN, et al. Effects of microgravity or simulated launch on testicular function in rats. *J Appl Physiol.* 1992;73:174S-85S.
870. Ortiz RM, Wang TJ, Wade CE. Influence of centrifugation and hindlimb suspension on testosterone and corticosterone excretion in rats. *Aviat Space Environ Med.* 1999;70:499-504.
871. Tash JS, Johnson DC, Enders GC. Long-term (6-wk) hindlimb suspension inhibits spermatogenesis in adult male rats. *J Appl Physiol.* 2002;92:1191-8.
872. Macho L, Kvetnansky R, Fickova M, Popova IA, Grigoriev A. Effects of exposure to space flight on endocrine regulations in experimental animals. *Endocr Regul.* 2001;35:101-14.
873. Royland JE, Weber LJ, Fitzpatrick M. Testes size and testosterone levels in a model for weightlessness. *Life Sci.* 1994;54:545-54.
874. Ricci G, Catizone A, Esposito R, Galdieri M. Microgravity effect on testicular functions. *J Gravit Physiol.* 2004;11:P61-2.
875. Ricci G, Esposito R, Catizone A, Galdieri M. Direct effects of microgravity on testicular function: analysis of histological, molecular and physiologic parameters. *J Endocrinol Invest.* 2008;31:229-37.
876. Smith BJ, King JB, Lucas EA, Akhter MP, Arjmandi BH, Stoecker BJ. Skeletal unloading and dietary copper depletion are detrimental to bone quality of mature rats. *J Nutr.* 2002;132:190-6.
877. Clarke BL, Khosla S. Androgens and bone. *Steroids.* 2009;74:296-305.
878. Sheffield-Moore M, Dillon EL, Casperson SL, Gilkison CR, Paddon-Jones D, Durham WJ, et al. A randomized pilot study of monthly cycled testosterone replacement or continuous testosterone replacement versus placebo in older men. *J Clin Endocrinol Metab.* 2011;96:E1831-7.
879. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab.* 2004;89:4351-8.
880. Brooks N, Cloutier GJ, Cadena SM, Layne JE, Nelsen CA, Freed AM, et al. Resistance training and timed essential amino acids protect against the loss of muscle mass and strength during 28 days of bed rest and energy deficit. *J Appl Physiol.* 2008;105:241-8.
881. Trappe TA, Burd NA, Louis ES, Lee GA, Trappe SW. Influence of concurrent exercise or nutrition countermeasures on thigh and calf muscle size and function during 60 days of bed rest in women. *Acta Physiol (Oxf).* 2007;191:147-59.
882. Antonione R, Caliendo E, Zorat F, Guarnieri G, Heer M, Biolo G. Whey protein ingestion enhances postprandial anabolism during short-term bed rest in young men. *J Nutr.* 2008;138:2212-6.
883. Brooks NE, Cadena SM, Vannier E, Cloutier G, Carambula S, Myburgh KH, et al. Effects of resistance exercise combined with essential amino acid supplementation and energy deficit on markers of skeletal muscle atrophy and regeneration during bed rest and active recovery. *Muscle Nerve.* 2010;42:927-35.
884. Ferrando AA, Williams BD, Stuart CA, Lane HW, Wolfe RR. Oral branched-chain amino acids decrease whole-body proteolysis. *JPEN J Parenter Enteral Nutr.* 1995;19:47-54.
885. Stein TP, Blanc S. Does protein supplementation prevent muscle disuse atrophy and loss of strength? *Crit Rev Food Sci Nutr.* 2011;51:828-34.
886. Kerstetter JE, Looker AC, Insogna KL. Low dietary protein and low bone density. *Calcif Tissue Int.* 2000;66:313.
887. Kerstetter JE, O'Brien KO, Insogna KL. Low protein intake: the impact on calcium and bone homeostasis in humans. *J Nutr.* 2003;133:855S-61S.
888. Kerstetter JE, Svastisalee CM, Caseria DM, Mitnick ME, Insogna KL. A threshold for low-protein-diet-induced elevations in parathyroid hormone. *Am J Clin Nutr.* 2000;72:168-73.
889. Norsk P. Blood pressure regulation IV: adaptive responses to weightlessness. *Eur J Appl Physiol.* 2014;114:481-97.
890. Norsk P, Damgaard M, Petersen L, Gybel M, Pump B, Gabrielsen A, et al. Vasorelaxation in space. *Hypertension.* 2006;47:69-73.

891. Aubert AE, Beckers F, Verheyden B. Cardiovascular function and basics of physiology in microgravity. *Acta Cardiol.* 2005;60:129-51.
892. Zhang LF. Region-specific vascular remodeling and its prevention by artificial gravity in weightless environment. *Eur J Appl Physiol.* 2013;113:2873-95.
893. Moore AD, Lee SMC, Stenger MB, Platts SH. Cardiovascular exercise in the U.S. space program: past, present, and future. *Acta Astronaut.* 2010;66:974-88.
894. Lee SM, Moore AD, Everett ME, Stenger MB, Platts SH. Aerobic exercise deconditioning and countermeasures during bed rest. *Aviat Space Environ Med.* 2010;81:52-63.
895. Iwasaki K, Levine BD, Zhang R, Zuckerman JH, Pawelczyk JA, Diedrich A, et al. Human cerebral autoregulation before, during and after spaceflight. *J Physiol.* 2007;579:799-810.
896. Nie ZL, Wang ZM, Zhou B, Tang ZP, Wang SK. Magnesium intake and incidence of stroke: meta-analysis of cohort studies. *Nutr Metab Cardiovasc Dis.* 2013;23:169-76.
897. Rowe WJ. As with space flight, a magnesium deficit may be responsible for both peripheral vascular dysfunction and kidney disease. *Am J Cardiol.* 2010;105:1203-4.
898. Volpe SL. Magnesium in disease prevention and overall health. *Adv Nutr.* 2013;4:378S-83S.
899. Bellavia A, Larsson SC, Bottai M, Wolk A, Orsini N. Fruit and vegetable consumption and all-cause mortality: a dose-response analysis. *Am J Clin Nutr.* 2013;98:454-9.
900. Leenders M, Sluijs I, Ros MM, Boshuizen HC, Siersema PD, Ferrari P, et al. Fruit and vegetable consumption and mortality: European prospective investigation into cancer and nutrition. *Am J Epidemiol.* 2013;178:590-602.
901. Dalen JE, Devries S. Diets to prevent coronary heart disease 1957-2013: what have we learned? *Am J Med.* 2014;127:364-9.
902. Appleby PN, Allen NE, Key TJ. Diet, vegetarianism, and cataract risk. *Am J Clin Nutr.* 2011.
903. Appleby P, Roddam A, Allen N, Key T. Comparative fracture risk in vegetarians and nonvegetarians in EPIC-Oxford. *Eur J Clin Nutr.* 2007;61:1400-6.
904. Mayasnikov VI, Stepanova SI. Features of cerebral hemodynamics in cosmonauts before and after flight on the Mir Orbital Station. *Orbital Station Mir Institute of Biomedical Problems: State Scientific Center of Russian Federation;* 2008. p. 300-5.
905. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA.* 1995;274:1526-33.
906. Finkelstein JD. The metabolism of homocysteine: pathways and regulation. *Eur J Pediatr.* 1998;157 Suppl 2:S40-4.
907. Pizza V, Agresta A, Agresta A, Lamaida E, Lamaida N, Infante F, et al. Migraine and genetic polymorphisms: an overview. *Open Neurol J.* 2012;6:65-70.
908. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA.* 2002;288:2015-22.
909. Ebrahimi KB, Handa JT. Lipids, lipoproteins, and age-related macular degeneration. *J Lipids.* 2011;2011 Jul 28. doi: 10.1155/2011/802059:802059.
910. Burgansky-Eliash Z, Barash H, Nelson D, Grinvald A, Sorkin A, Loewenstein A, et al. Retinal blood flow velocity in patients with age-related macular degeneration. *Curr Eye Res.* 2014;39:304-11.
911. Evans J. Should we be taking B vitamins to prevent age-related macular degeneration? Not yet, but worth doing more research. *Am J Clin Nutr.* 2013;98:4-5.
912. Sekeryapan B, Oner V, Kirbas A, Turkyilmaz K, Durmus M. Plasma homocysteine levels in dry eye patients. *Cornea.* 2013;32:e94-6.
913. Xu F, Zhao X, Zeng SM, Li L, Zhong HB, Li M. Homocysteine, B vitamins, methylenetetrahydrofolate reductase gene, and risk of primary open-angle glaucoma: a meta-analysis. *Ophthalmology.* 2012;119:2493-9.
914. Rochtchina E, Wang JJ, Flood VM, Mitchell P. Elevated serum homocysteine, low serum vitamin B12, folate, and age-related macular degeneration: the Blue Mountains Eye Study. *Am J Ophthalmol.* 2007;143:344-6.
915. Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the Women's Antioxidant and Folic Acid Cardiovascular Study. *Arch Intern Med.* 2009;169:335-41.
916. Blanchflower DG, Oswald AJ, Stewart-Brown S. Is psychological well-being linked to the consumption of fruit and vegetables? *Social Indicators Res.* 2013;114:785-801.
917. Gomez-Pinilla F. Brain foods: the effects of nutrients on brain function. *Nat Rev Neurosci.* 2008;9:568-78.

- 918.Chennaoui M, Desgorges F, Drogou C, Boudjemaa B, Tomaszewski A, Depiesse F, et al. Effects of Ramadan fasting on physical performance and metabolic, hormonal, and inflammatory parameters in middle-distance runners. *Appl Physiol Nutr Metab.* 2009;34:587-94.
- 919.Nabb S, Benton D. The influence on cognition of the interaction between the macro-nutrient content of breakfast and glucose tolerance. *Physiol Behav.* 2006;87:16-23.
- 920.Nabb SL, Benton D. The effect of the interaction between glucose tolerance and breakfasts varying in carbohydrate and fibre on mood and cognition. *Nutr Neurosci.* 2006;9:161-8.
- 921.Benton D, Nabb S. Carbohydrate, memory, and mood. *Nutr Rev.* 2003;61:S61-7.
- 922.Fuller PM, Lu J, Saper CB. Differential rescue of light- and food-entrainable circadian rhythms. *Science.* 2008;320:1074-7.
- 923.Li JJ, Huang ZW, Wang RQ, Ma XM, Zhang ZQ, Liu Z, et al. Fruit and vegetable intake and bone mass in Chinese adolescents, young and postmenopausal women. *Public Health Nutr.* 2013;16:78-86.
- 924.Rooney C, McKinley MC, Woodside JV. The potential role of fruit and vegetables in aspects of psychological well-being: a review of the literature and future directions. *Proc Nutr Soc.* 2013;72:420-32.
- 925.Stranges S, Samaraweera PC, Taggart F, Kandala NB, Stewart-Brown S. Major health-related behaviours and mental well-being in the general population: the Health Survey for England. *BMJ Open.* 2014;4:e005878.
- 926.Myint PK, Welch AA, Bingham SA, Surtees PG, Wainwright NW, Luben RN, et al. Fruit and vegetable consumption and self-reported functional health in men and women in the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk): a population-based cross-sectional study. *Public Health Nutr.* 2007;10:34-41.
- 927.Jacka FN, Pasco JA, Mykletun A, Williams LJ, Hodge AM, O'Reilly SL, et al. Association of Western and traditional diets with depression and anxiety in women. *Am J Psychiatry.* 2010;167:305-11.
- 928.Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience.* 2002;112:803-14.
- 929.Stuster J. Behavioral issues associated with long-duration space expeditions: Review and analysis of astronaut journals. Experiment 01-E104 (Journals): Final Report (NASA/TM-2010-216130). Houston, TX: National Aeronautics and Space Administration Johnson Space Center2010.
- 930.Nilsson A, Radeborg K, Salo I, Bjorck I. Effects of supplementation with n-3 polyunsaturated fatty acids on cognitive performance and cardiometabolic risk markers in healthy 51 to 72 years old subjects: a randomized controlled cross-over study. *Nutr J.* 2012;11:99.
- 931.van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr.* 2007;85:1142-7.
- 932.Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, et al. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *J Clin Psychiatry.* 2006;67:1954-67.
- 933.Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J Neurotrauma.* 2004;21:1457-67.
- 934.Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. *J Nutr.* 2005;135:549-55.
- 935.Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron.* 2004;43:633-45.
- 936.Danthiir V, Hosking D, Burns NR, Wilson C, Nettelbeck T, Calvaresi E, et al. Cognitive performance in older adults is inversely associated with fish consumption but not erythrocyte membrane n-3 fatty acids. *J Nutr.* 2014;144:311-20.
- 937.Cole GM, Lim GP, Yang F, Teter B, Begum A, Ma Q, et al. Prevention of Alzheimer's disease: Omega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol Aging.* 2005;26 Suppl 1:133-6.
- 938.Wu A, Ying Z, Gomez-Pinilla F. Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative stress, synaptic plasticity, and cognition. *Exp Neurol.* 2006;197:309-17.
- 939.van Praag H, Lucero MJ, Yeo GW, Stecker K, Heivand N, Zhao C, et al. Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J Neurosci.* 2007;27:5869-78.
- 940.Lettenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol.* 2007;165:1364-71.

941. Wu A, Molteni R, Ying Z, Gomez-Pinilla F. A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. *Neuroscience*. 2003;119:365-75.
942. Greenwood CE, Winocur G. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol Aging*. 2005;26 Suppl 1:42-5.
943. Moorthy D, Peter I, Scott TM, Parnell LD, Lai CQ, Crott JW, et al. Status of vitamins B-12 and B-6 but not of folate, homocysteine, and the methylenetetrahydrofolate reductase C677T polymorphism are associated with impaired cognition and depression in adults. *J Nutr*. 2012;142:1554-60.
944. Bryan J, Calvaresi E, Hughes D. Short-term folate, vitamin B-12 or vitamin B-6 supplementation slightly affects memory performance but not mood in women of various ages. *J Nutr*. 2002;132:1345-56.
945. Chang N, Kim E, Kim KN, Kim H, Kim SY, Jeong BS. Folate nutrition is related to neuropsychological functions in the elderly. *Nutr Res Pract*. 2009;3:43-8.
946. Sasaki H, Matsuzaki Y, Meguro K, Ikarashi Y, Maruyama Y, Yamaguchi S, et al. Vitamin B12 improves cognitive disturbance in rodents fed a choline-deficient diet. *Pharmacol Biochem Behav*. 1992;43:635-9.
947. Calvaresi E, Bryan J. B vitamins, cognition, and aging: a review. *J Gerontol B Psychol Sci Soc Sci*. 2001;56:P327-39.
948. Deijen JB, van der Beek EJ, Orlebeke JF, van den Berg H. Vitamin B-6 supplementation in elderly men: effects on mood, memory, performance and mental effort. *Psychopharmacology (Berl)*. 1992;109:489-96.
949. Eussen SJ, de Groot LC, Joosten LW, Bloo RJ, Clarke R, Ueland PM, et al. Effect of oral vitamin B-12 with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial. *Am J Clin Nutr*. 2006;84:361-70.
950. Przybelski RJ, Binkley NC. Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. *Arch Biochem Biophys*. 2007;460:202-5.
951. Annweiler C, Allali G, Allain P, Bridenbaugh S, Schott AM, Kressig RW, et al. Vitamin D and cognitive performance in adults: a systematic review. *Eur J Neurol*. 2009;16:1083-9.
952. Constans T, Mondon K, Annweiler C, Hommet C. [Vitamin D and cognition in the elderly]. *Psychol Neuropsychiatr Vieil*. 2010;8:255-62.
953. Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *Eur J Neurosci*. 2004;19:1699-707.
954. Perkins AJ, Hendrie HC, Callahan CM, Gao S, Unverzagt FW, Xu Y, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. 1999;150:37-44.
955. Holmes GL, Yang Y, Liu Z, Cermak JM, Sarkisian MR, Stafstrom CE, et al. Seizure-induced memory impairment is reduced by choline supplementation before or after status epilepticus. *Epilepsy Res*. 2002;48:3-13.
956. McCann JC, Hudes M, Ames BN. An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring. *Neurosci Biobehav Rev*. 2006;30:696-712.
957. Wengreen HJ, Munger RG, Corcoran CD, Zandi P, Hayden KM, Fotuhi M, et al. Antioxidant intake and cognitive function of elderly men and women: the Cache County Study. *J Nutr Health Aging*. 2007;11:230-7.
958. Schram MT, Trompet S, Kamper AM, de Craen AJ, Hofman A, Euser SM, et al. Serum calcium and cognitive function in old age. *J Am Geriatr Soc*. 2007;55:1786-92.
959. Gao S, Jin Y, Unverzagt FW, Liang C, Hall KS, Cao J, et al. Selenium level and depressive symptoms in a rural elderly Chinese cohort. *BMC*. 2012. doi: 10.1186/1471-244X-12-72;12:72.
960. Gao S, Jin Y, Hall KS, Liang C, Unverzagt FW, Ji R, et al. Selenium level and cognitive function in rural elderly Chinese. *Am J Epidemiol*. 2007;165:955-65.
961. Pajonk FG, Kessler H, Supprian T, Hamzei P, Bach D, Schweickhardt J, et al. Cognitive decline correlates with low plasma concentrations of copper in patients with mild to moderate Alzheimer's disease. *J Alzheimers Dis*. 2005;8:23-7.
962. Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. *Am J Clin Nutr*. 2007;85:778-87.
963. Power SE, O'Connor EM, Ross RP, Stanton C, O'Toole PW, Fitzgerald GF, et al. Dietary glycaemic load associated with cognitive performance in elderly subjects. *Eur J Nutr*. 2014 Jul 18 [Epub ahead of print].

964. Bresnahan KA, Tanumihardjo SA. Undernutrition, the Acute Phase Response to Infection, and Its Effects on Micronutrient Status Indicators. *Adv Nutr.* 2014;5:702-11.
965. Beisel WR. History of nutritional immunology: introduction and overview. *J Nutr.* 1992;122:591-6.
966. Mizock BA. Immunonutrition and critical illness: an update. *Nutrition.* 2010;26:701-7.
967. Cunningham-Rundles S, McNeeley DF, Moon A. Mechanisms of nutrient modulation of the immune response. *J Allergy Clin Immunol.* 2005;115:1119-28; quiz 29.
968. Chandra RK, Kumari S. Effects of nutrition on the immune system. *Nutrition.* 1994;10:207-10.
969. Chandra RK. Nutrient regulation of immune functions. *Forum Nutr.* 2003;56:147-8.
970. Chandra RK. Nutrition and the immune system: an introduction. *Am J Clin Nutr.* 1997;66:460S-3S.
971. Keith ME, Jeejeebhoy KN. Immunonutrition. *Baillieres Clin Endocrinol Metab.* 1997;11:709-38.
972. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab.* 2007;51:301-23.
973. Sonnenfeld G. The immune system in space, including Earth-based benefits of space-based research. *Curr Pharm Biotechnol.* 2005;6:343-9.
974. Sonnenfeld G, Shearer WT. Immune function during space flight. *Nutrition.* 2002;18:899-903.
975. Stein TP, Gairindashvili T. Spaceflight and protein metabolism, with special reference to humans. *Am J Clin Nutr.* 1994;60:806S-19S.
976. Chandra RK, Chandra S, Gupta S. Antibody affinity and immune complexes after immunization with tetanus toxoid in protein-energy malnutrition. *Am J Clin Nutr.* 1984;40:131-4.
977. Guadagni M, Biolo G. Effects of inflammation and/or inactivity on the need for dietary protein. *Curr Opin Clin Nutr Metab Care.* 2009;12:617-22.
978. Kirk SJ, Barbul A. Role of arginine in trauma, sepsis, and immunity. *JPEN J Parenter Enteral Nutr.* 1990;14:226S-9S.
979. Kirk SJ, Hurson M, Regan MC, Holt DR, Wasserkrug HL, Barbul A. Arginine stimulates wound healing and immune function in elderly human beings. *Surgery.* 1993;114:155-9; discussion 60.
980. Singleton KD, Wischmeyer PE. Glutamine attenuates inflammation and NF-kappaB activation via Cullin-1 deneddylation. *Biochem Biophys Res Commun.* 2008;373:445-9.
981. Wischmeyer PE. Glutamine: role in critical illness and ongoing clinical trials. *Curr Opin Gastroenterol.* 2008;24:190-7.
982. Wischmeyer PE. Glutamine: mode of action in critical illness. *Crit Care Med.* 2007;35:S541-4.
983. Mondello S, Italiano D, Giacobbe MS, Mondello P, Trimarchi G, Aloisi C, et al. Glutamine-supplemented total parenteral nutrition improves immunological status in anorectic patients. *Nutrition.* 2010;26:677-81.
984. Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit Care Med.* 2002;30:2022-9.
985. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol.* 2005;97:93-101.
986. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med.* 2002;8:174-9.
987. Hewison M, Freeman L, Hughes SV, Evans KN, Bland R, Eliopoulos AG, et al. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. *J Immunol.* 2003;170:5382-90.
988. Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, Skolimowska K, et al. IFN-gamma- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. *J Immunol.* 2007;178:7190-8.
989. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, et al. A single dose of vitamin D enhances immunity to mycobacteria. *Am J Respir Crit Care Med.* 2007;176:208-13.
990. Kondo Y, Kato T, Kimura O, Iwata T, Ninomiya M, Kakazu E, et al. 1(OH) vitamin D3 supplementation improves the sensitivity of the immune-response during Peg-IFN/RBV therapy in chronic hepatitis C patients-case controlled trial. *PLoS One.* 2013;8:e63672.
991. Chouker A. Stress challenges and immunity in space: from mechanisms to monitoring and preventive strategies. Berlin: Springer; 2012.
992. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol.* 1999;116:28-32.

993. Bunout D, Barrera G, Hirsch S, Gattas V, de la Maza MP, Haschke F, et al. Effects of a nutritional supplement on the immune response and cytokine production in free-living Chilean elderly. *JPEN J Parenter Enteral Nutr.* 2004;28:348-54.
994. Maggini S, Wintergerst ES, Beveridge S, Hornig DH. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *Br J Nutr.* 2007;98 Suppl 1:S29-35.
995. Heer M, Baisch F, Kropp J, Gerzer R, Drummer C. High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am J Physiol Renal Physiol.* 2000;278:F585-95.
996. Titze J, Lang R, Ilies C, Schwind KH, Kirsch KA, Dietsch P, et al. Osmotically inactive skin Na<sup>+</sup> storage in rats. *Am J Physiol Renal Physiol.* 2003;285:F1108-17.
997. Marvar PJ, Gordon FJ, Harrison DG. Blood pressure control: salt gets under your skin. *Nat Med.* 2009;15:487-8.
998. Chen Q, Ross AC. Vitamin A and immune function: retinoic acid modulates population dynamics in antigen receptor and CD38-stimulated splenic B cells. *Proc Natl Acad Sci U S A.* 2005;102:14142-9.
999. Ross AC. Vitamin A and retinoic acid in T cell-related immunity. *Am J Clin Nutr.* 2012;96:1166S-72S.
1000. Ross AC. Vitamin A status: relationship to immunity and the antibody response. *Proc Soc Exp Biol Med.* 1992;200:303-20.
1001. Stephensen CB. Vitamin A, infection, and immune function. *Annu Rev Nutr.* 2001;21:167-92.
1002. Stephensen CB. Examining the effect of a nutrition intervention on immune function in healthy humans: what do we mean by immune function and who is really healthy anyway? *Am J Clin Nutr.* 2001;74:565-6.
1003. Semba RD. The role of vitamin A and related retinoids in immune function. *Nutr Rev.* 1998;56:S38-48.
1004. Field CJ, Johnson IR, Schley PD. Nutrients and their role in host resistance to infection. *J Leukoc Biol.* 2002;71:16-32.
1005. Schwager J, Schulze J. Modulation of interleukin production by ascorbic acid. *Vet Immunol Immunopathol.* 1998;64:45-57.
1006. Jeng KC, Yang CS, Siu WY, Tsai YS, Liao WJ, Kuo JS. Supplementation with vitamins C and E enhances cytokine production by peripheral blood mononuclear cells in healthy adults. *Am J Clin Nutr.* 1996;64:960-5.
1007. Park OJ, Kim HY, Kim WK, Kim YJ, Kim SH. Effect of vitamin E supplementation on antioxidant defense systems and humoral immune responses in young, middle-aged and elderly Korean women. *J Nutr Sci Vitaminol (Tokyo).* 2003;49:94-9.
1008. Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Ann Nutr Metab.* 2006;50:85-94.
1009. Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I. Regulation of SIRT1 in cellular functions: role of polyphenols. *Arch Biochem Biophys.* 2010;501:79-90.
1010. Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochem Biophys.* 2009;53:75-100.
1011. Gao X, Deeb D, Media J, Divine G, Jiang H, Chapman RA, et al. Immunomodulatory activity of resveratrol: discrepant in vitro and in vivo immunological effects. *Biochem Pharmacol.* 2003;66:2427-35.
1012. Park HJ, Lee CM, Jung ID, Lee JS, Jeong YI, Chang JH, et al. Quercetin regulates Th1/Th2 balance in a murine model of asthma. *Int Immunopharmacol.* 2009;9:261-7.
1013. Sharma S, Chopra K, Kulkarni SK, Agrewala JN. Resveratrol and curcumin suppress immune response through CD28/CTLA-4 and CD80 co-stimulatory pathway. *Clin Exp Immunol.* 2007;147:155-63.
1014. Shim JH, Choi HS, Pugliese A, Lee SY, Chae JI, Choi BY, et al. (-)-Epigallocatechin gallate regulates CD3-mediated T cell receptor signaling in leukemia through the inhibition of ZAP-70 kinase. *J Biol Chem.* 2008;283:28370-9.
1015. Singh NP, Hegde VL, Hofseth LJ, Nagarkatti M, Nagarkatti P. Resveratrol (trans-3,5,4'-trihydroxystilbene) ameliorates experimental allergic encephalomyelitis, primarily via induction of apoptosis in T cells involving activation of aryl hydrocarbon receptor and estrogen receptor. *Mol Pharmacol.* 2007;72:1508-21.
1016. Song EK, Hur H, Han MK. Epigallocatechin gallate prevents autoimmune diabetes induced by multiple low doses of streptozotocin in mice. *Arch Pharm Res.* 2003;26:559-63.
1017. Ganz T. Hepcidin--a peptide hormone at the interface of innate immunity and iron metabolism. *Curr Top Microbiol Immunol.* 2006;306:183-98.
1018. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood.* 2011;117:4425-33.

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1019. Lee P, Peng H, Gelbart T, Wang L, Beutler E. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proc Natl Acad Sci U S A*. 2005;102:1906-10.
1020. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113:1271-6.
1021. Vanamala J, Glagolenko A, Yang P, Carroll RJ, Murphy ME, Newman RA, et al. Dietary fish oil and pectin enhance colonocyte apoptosis in part through suppression of PPARdelta/PGE2 and elevation of PGE3. *Carcinogenesis*. 2008;29:790-6.
1022. Camandola S, Leonarduzzi G, Musso T, Varesio L, Carini R, Scavazza A, et al. Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem Biophys Res Commun*. 1996;229:643-7.
1023. Kang JX, Weylandt KH. Modulation of inflammatory cytokines by omega-3 fatty acids. *Subcell Biochem*. 2008;49:133-43.
1024. Kim HH, Lee Y, Eun HC, Chung JH. Eicosapentaenoic acid inhibits TNF-alpha-induced matrix metalloproteinase-9 expression in human keratinocytes, HaCaT cells. *Biochem Biophys Res Commun*. 2008;368:343-9.
1025. Magee P, Pearson S, Allen J. The omega-3 fatty acid, eicosapentaenoic acid (EPA), prevents the damaging effects of tumour necrosis factor (TNF)-alpha during murine skeletal muscle cell differentiation. *Lipids Health Dis*. 2008;7:24.
1026. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. *Nature*. 2011;474:327-36.
1027. David LA, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, et al. Host lifestyle affects human microbiota on daily timescales. *Genome Biol*. 2014;15:R89.
1028. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med*. 2009;1:6ra14.
1029. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. *Science*. 2006;312:1355-9.
1030. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science*. 2011;334:105-8.
1031. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc*. 1988;88:1268-71.
1032. Lane HW, Zwart SR, Kloeris V, Smith SM. Food and nutrition for space flight. In: Berdanier CD, Dwyer JT, Heber D, editors. *Handbook of Nutrition and Food*. 3rd ed. New York: CRC Press; 2013. p. 381-402.

## **XVIII. Team**

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**XIX. Appendix A. Nutrient Requirements and Dietary Intake Data Tables**

**Table 1.** Planned (menu) and targeted nutrient intake on International Space Station missions.<sup>1</sup>

	Menu Content <sup>2</sup>	NASA Spaceflight Requirement (5)
Energy, kcal/d	2877 ± 167 <sup>3</sup>	Based on WHO (27)
Energy, % WHO	99 ± 13	
Total carbohydrate, % of kcal	50 ± 3	50-55
Total protein, g/d	126 ± 10	
Total protein, % of kcal	17 ± 1	12-15
Animal protein, g/d	72 ± 7	60%
Vegetable protein, g/d	33 ± 3	40%
Total fat, % of kcal	31 ± 1	30-35
Total dietary fiber, g/d	33 ± 4	10-25
Retinol equivalents, µg/d	1420 ± 205	1000
Vitamin D, µg/d	4.2 ± 1.0	10
Vitamin E (total α-tocopherol equivalents), mg/d	12.1 ± 1.9	20
Vitamin K (phylloquinone), µg/d	105 ± 19	80
Vitamin C (ascorbic acid), mg/d	191 ± 39	100
Thiamin, mg/d	2.0 ± 0.1	1.5
Riboflavin, mg/d	2.2 ± 0.2	2.0
Niacin, mg/d	29.8 ± 1.9	20 mg niacin equivalents
Pantothenic acid, mg/d	5.1 ± 0.8	5.0
Vitamin B <sub>6</sub> , mg/d	2.3 ± 0.2	2.0
Total folate, µg/d	434 ± 53	400
Vitamin B <sub>12</sub> (cobalamin), µg/d	4.6 ± 0.7	2.0
Calcium, mg/d	1020 ± 109	1000-1200
Phosphorus, mg/d	1856 ± 165	1000-1200 (NTE 1.5 x Ca)
Phosphorus:calcium ratio	1.83 ± 0.17	<1.5
Magnesium, mg/d	424 ± 40	350
Iron, mg/d	22.7 ± 4.5	10
Copper, mg/d	3.6 ± 0.9	1.5-3.0
Zinc, mg/d	22.1 ± 6.2	15
Manganese, mg/d	5.7 ± 0.7	2-5
Selenium, µg/d	146 ± 16	70
Iodine, mg/d	1.0 ± 2.8	0.15
Sodium, mg/d	5625 ± 531	<3500
Potassium, mg/d	3995 ± 360	3500
Water, g/d	2155 ± 206	1 mL/kcal, > 2 liters per day

WHO, World Health Organization.

<sup>1</sup>Table adapted from Smith and Zwart (1).

<sup>2</sup>Menu data are derived from either proximate analysis of space foods (macronutrients, most minerals) or estimations (animal protein, vegetable protein, all vitamins, selenium) from similar items in the Nutrition Data System for Research (NDS-R) database, versions 4.03/31, 4.05/33, 4.06/34, 5.0/35, 2005, and 2006, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA (1031).

<sup>3</sup>All data are the mean ± SD and represent the average from menus of 19 ISS astronauts.

Table 2. 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.

	<b>Apollo</b>	<b>Skylab</b>	<b>Shuttle</b>	<b>ISS (E1-13)</b>	<b>ISS (E14-25)</b>	<b>ISS (E26-37)</b>
N	33	9	32	19	19	17
Energy, kcal/d	1880 ± 415 <sup>a</sup>	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 <sup>b</sup>	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.

<sup>a</sup>All data are mean ± SD. Empty cells show where data were not available. Data updated and expanded from earlier reports (2, 1032).

<sup>b</sup>N=3 for water intake during Apollo missions.

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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 ..... 170

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**XXI. Appendix C. Abbreviations**

8OHdG	8-hydroxy-2'-deoxyguanosine
ACP	acyl carrier protein
ADP	adenosine diphosphate
AI	adequate intake
AME	annual medical examination
AMP	adenosine monophosphate
ARED	Advanced Resistance Exercise Device
BDNF	brain-derived neurotrophic factor
BHP	Behavioral Health and Performance Element (of the Human Research Program)
BMD	bone mineral density
BMMD	Body Mass Measuring Device
BSAP	bone-specific alkaline phosphatase
cal	calorie
CoA	coenzyme A
d	day
DFE	dietary folate equivalent(s)
DLR	German Aerospace Center
DNA	deoxyribonucleic acid
DRI	dietary reference intake
DXA	dual-energy x-ray absorptiometry
EBV	Epstein-Barr virus
EER	estimated energy requirements
EGR	erythrocyte glutathione reductase
Eq	equivalent
ESA	European Space Agency
EVA	extravehicular activity (space walk)
FAD	flavin adenine dinucleotide
FD	flight day
g	gram
<i>g</i>	acceleration due to gravity (1 <i>g</i> = Earth gravity)
GLA	$\gamma$ -carboxyglutamic acid
GPX	glutathione peroxidase
Gy	Gray
h, hr	hour
HDL	high-density lipoprotein
HRP	Human Research Program
Hz	hertz
IFN	interferon
IL	interleukin
IOM	Institute of Medicine
iRED	interim resistance exercise device
ISS	International Space Station
IU	international unit

*Risk Factor of Inadequate Nutrition*

J	joule
K	kilo
KCit	potassium citrate
L	liter
LDL	low-density lipoprotein
LBNP	lower-body negative pressure
LH	luteinizing hormone
μ	micro
m	meter, milli
MCA	methylcitric acid
MDA	malondialdehyde
MMA	methylmalonic acid
mol	mole
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
NE	niacin equivalent
NEEMO	NASA Extreme Environment Mission Operations
NF-κB	NF-kappa B protein complex, which functions as a nuclear transcription factor
NK	natural killer
NTX	n-telopeptide
<i>P</i>	probability
PGF2α	8-iso-prostaglandin F2α
PL	pyridoxal
PLP	pyridoxal 5'-phosphate
PM	pyridoxamine
PMP	pyridoxamine 5'-phosphate
PN	pyridoxine
PNP	pyridoxine 5'-phosphate
psia	pound(s) per square inch absolute
PTH	parathyroid hormone
PUFA	polyunsaturated fatty acid
<i>r</i>	bivariate correlation coefficient
R+0	0 days after landing (recovery)
RBC	red blood cell
RDA	recommended dietary allowance
RE	retinol equivalent
RNA	ribonucleic acid
ROS	reactive oxygen species
SAM	S-adenosylmethionine
SD	standard deviation
SLAMMD	Space Linear Acceleration Mass Measuring Device
SMO	Supplemental Medical Objective

*Risk Factor of Inadequate Nutrition*

TEE	total energy expenditure
THF	tetrahydrofolate
TPP	thiamin pyrophosphate
U	unit
ULLS	unilateral limb suspension
UPA	Urine Processor Assembly
UV-B	ultraviolet B
VDR	vitamin D receptor
WHO	World Health Organization
y	year