Minireview

Impacts of microgravity on amino acid metabolism during spaceflight

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Impact Statement

A significant loss of body protein, impaired blood flow, and oxidative stress are significant problems for astronauts and space travelers who experience microgravity. This review highlights advances in findings on the effects of microgravity on amino acid metabolism in various tissues of astronauts. The article also underscores the importance of functional amino acids in stimulating protein synthesis and reducing proteolysis in skeletal muscle, as well as alleviating oxidative stress and vascular abnormalities under these conditions. Such knowledge provides rationale for future research to mitigate adverse effects of spaceflight on muscle protein balance and improve the health of astronauts. This will further aid in the successful development of long-term manned space mission and permanent space habitats.

Abstract

Spaceflight exerts an extreme and unique influence on human physiology as astronauts are subjected to long-term or short-term exposure to microgravity. During spaceflight, a multitude of physiological changes, including the loss of skeletal muscle mass, bone resorption, oxidative stress, and impaired blood flow, occur, which can affect astronaut health and the likelihood of mission success. *In vivo* and *in vitro* metabolite studies suggest that amino acids are among the most affected nutrients and metabolites by microgravity (a weightless condition due to very weak gravitational forces). Moreover, exposure to microgravity alters gut microbial composition, immune function, musculoskeletal health, and consequently amino acid metabolism. Appropriate knowledge of daily protein consumption, with a focus on specific functional amino acids, may offer insight into potential combative and/or therapeutic effects of amino acid consumption in astronauts and space travelers. This will further aid in the successful development of long-term manned space mission and permanent space habitats.

Keywords: Protein, immune response, nutrition, astronaut, skeletal muscle

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Introduction

Dietary intake is an integral component of astronaut health and functionality during spaceflight and for successful performance of extravehicular activities (EVAs). Food intake and its role on human spaceflight adaptation is a topic of extensive research and has been eloquently discussed by Smith *et al*.[1] Even so, in the coming years as humans seek to venture back to the moon and beyond, spaceflight mission success will hinge upon optimal dietary intake, including amino acids. In the wake of an extended exposure to microgravity (a weightless condition due to very weak gravitational forces), the loss of protein and nitrogen in astronauts has been consistently found, necessitating the consumption of amino acids especially for skeletal muscle health and function.[2] Amino acids are not simply the building blocks of proteins and peptides, they are signaling and regulatory molecules in whole-body metabolism as well.[3] Across species, a dietary deficiency of amino acids can lead to pathological conditions or maladaptive physiological responses.[4–7] Thus, it is crucial for organisms to meet nutritional needs for amino acids.

Other than actual spaceflight, experimentally it is possible to mimic microgravity in humans, animals, and cells in ground-based research. Methods, such as head-down tilt bed rest, dry and wet immersion, unilateral leg suspension, and limb immobilization in humans, have been used to study effects of microgravity on physiology.[8–11] In addition, hindlimb unloading in rodents has been employed to assess similar responses.[12] Furthermore, microgravity can be mimicked in cell samples with two-dimensional and three-dimensional clinorotation as the method characterizes small centrifugal forces about a rotating axis similar to the centrifugal forces experienced in a lack of gravity.[13]

During spaceflight, individuals are susceptible to a wide variety of deleterious conditions such as radiation exposure,

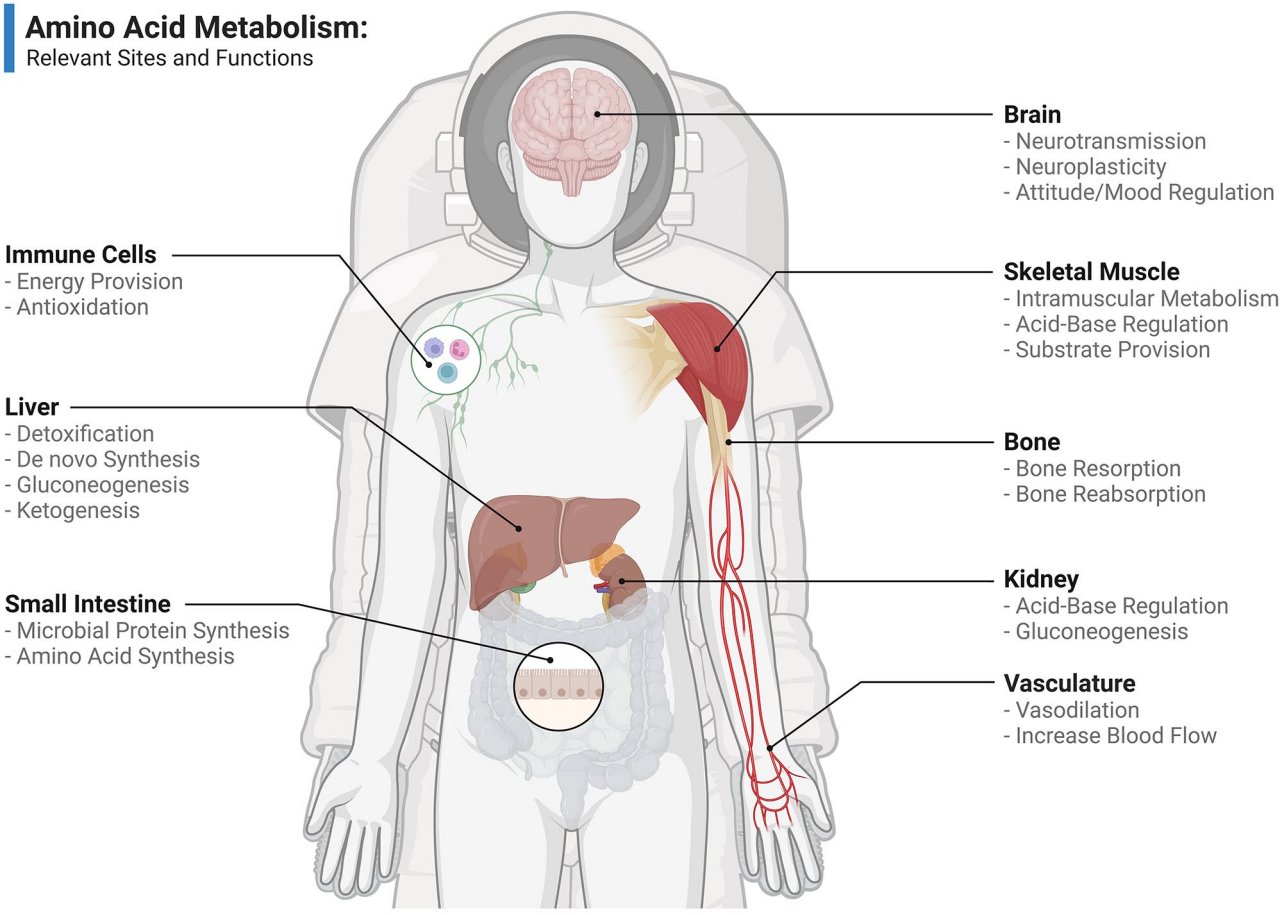

Figure 1. Amino acid metabolism: Relevant sites and functions.
An overview of key sites in the body for amino acid metabolism and some functionalities it impacts.

loss of muscle mass, increased bone resorption, immune system deregulation, ocular damage from increased cranial pressure, hypovolemia, cardiovascular deconditioning, and impaired blood flow.[2,14–17] However, adequate intakes of nutritionally essential amino acids (EAAs) and the traditionally classified nutritionally non-essential amino acids (NEAAs) can help combat the onset of these conditions.[15,17] The importance of EAAs has been well established, but recent research has suggested NEAAs possess a much greater functionality than previously thought and even proposes a new nomenclature for certain NEAAs, amino acids synthesizable de novo in animal cells (AASAs).[3,7,18] Hence, the consumption of "NEAAs" should be more strongly considered within the context of astronaut health. This review will highlight the metabolism of amino acids and their importance in astronaut health and function, the multi-systemic roles of amino acids in health and vitality, and recommendations for astronaut protein intake.

Digestion and degradation of amino acids

Amino acid metabolism is a systemic process comprising both synthesis and degradation, requiring coordinated communication between multiple organs and systems. This interorgan communication involves the metabolism and transport of amino acids to and from multiple sites in the body such as the small intestine, liver, skeletal muscle, kidneys, brain, and blood. Figure 1 depicts multiple sites and organs where amino acid metabolism is regulated. That said, we will start by discussing protein digestion following consumption, the subsequent degradation and transport of amino acids, and the effects microgravity exerts on amino acid metabolism in the gut.

To be physiologically useful, proteins must first be hydrolyzed into constituent amino acids. This process is initiated by matrix metalloproteinases in the saliva before quickly entering the stomach to begin proteolysis.[19,20] Protein degradation occurs to a limited extent in the stomach prior to the small intestine, the lumen of which being the primary contributor overall.[20] Enterocyte- and pancreas-derived proteases and peptidases in the small intestine hydrolyze proteins and large peptides ultimately generating free amino acids, dipeptides, and tripeptides.[20] Di- and tripeptides, as well as free amino acids, are transported into the enterocyte, where those that are not degraded or used by the cell exit the enterocyte via its basolateral membrane.[3] An important note is that amino acids can be degraded at various rates in the small intestine, through first-pass metabolism, such that the amount released from dietary protein entering portal circulation ranges from 4% to 85%.[3] Glutamine, glutamate, and aspartate in diets are extensively degraded by the mucosa of

Table 1. Changes in the gut and microbiota affecting protein and amino acid metabolism.

Model	Intervention	Sample	Significant Findings	Reference
Human				
Male volunteers (7 people)	45-day head-down tilt bed rest (HDBR)	Feces	• Decline in *Lactobacillus* and *Bifidobacterium* spp.	Chen *et al*.[9]
Identical twins (2 people)	Twin #1 – Earthbound Twin #2 – 340 days on ISS	Feces	• Flight altered gut microbiota composition and AA profile	Garret-Bakelman *et al*.[27]
Crew members (3 people)	105-day high-plant and high-fiber diet at Chinese Lunar Palace	Feces	• Increased Firmicutes/Bacteroidetes ratio • Increased diversity of Lachnospira, Faecalibacterium, and Blautia affected by lifestyle and dietary habits	Hao *et al*.[29]
Animal				
Wistar rats (24 rats)	Control, 14, or 21 days Tail suspension [hindlimb unloading]	Jejunum	• Reduced tight junction protein expression vs. controls	Ying *et al*.[26]
Cell				
E. coli	398 h on Shenzhou VIII vs. Ground controls	GGMCC 1.2385 LCT-EC226 LCT-EC67	• 167 proteins overexpressed and 92 downregulated post-flight: affecting alanine, glutamate, arginine, proline, fatty acid, and propanoate metabolism	Zhang *et al*.[28]
E. coli	Cells onboard STS-37, -43, -50, -54, -57, -60, -62 fights	ATCC 4157	• 88% greater bacterial cell growth over control cells	Klaus *et al*.[30]

the small intestine (about 70–96%) during first-pass metabolism, and these three amino acids are synthesized mainly by extraintestinal tissues, such as the skeletal muscle, liver, heart, and white adipose tissue. Dietary intakes of glutamine, glutamate, and aspartate are often overlooked in nutrition research despite the evidence on the importance of their consumption to yield optimal health benefits.[21] Arginine and proline are degraded to a lesser extent (about 40%) during first-pass metabolism, indicating they must be endogenously synthesized by tissues.[22,23] In the small intestine, branched-chain amino acids (BCAAs) and other EAAs are not as extensively catabolized as arginine and proline.[3] Yet, BCAAs are important nitrogenous precursors because they carry the amino group necessary for amino acid synthesis.[24]

After amino acids enter the portal vein, they can be transported systemically to organs and tissues for utilization. Some amino acids (i.e. citrulline) bypass the liver due to the lack of transmembrane transporters, whereas other amino acids (e.g. arginine) are taken up by the liver at a low rate (about 8% to 10%). An interorgan communication network allows for transport of amino acids to and from organs. This is important for multi-systemic functionality and health, as amino acids are involved in whole-body homeostasis. Proteinogenic amino acids are oxidized to either pyruvate, acetyl-CoA, or both in a tissue-specific manner.[3] Many other metabolites of amino acids, such as nitric oxide (NO), creatine, and polyamines, have enormous physiological roles in humans.[25]

It has been noted that digestive function is altered in microgravity. Table 1 lists studies that found effects of microgravity exposure *in vitro* and *in vivo* on gut bacteria, which may translate into alterations in the amino acid profile by affecting amino acid utilization by the gut.[26–30] One possibility is that an increased secretion of salivary matrix metalloproteinases may alter how dietary proteins are digested once they reach the gastrointestinal tract in humans under the conditions of a simulated microgravity.[31] Mouse models of simulated weightlessness have reported that the expression of tight junction proteins between epithelial cells of the small intestine was decreased, which could potentially promote endotoxemia and result in a decreased transcellular absorption of amino acids.[26] For example, some peptides with four or more amino acid residues appear to be absorbed via the tight junctions in earthbound humans, and a decrease in tight junction protein expression hinders tight junction integrity.[26]

Gut microbial composition is also altered during spaceflight of various durations and in simulated microgravity in humans, rodents, and cells.[27,29,32–34] Radiation exposure during spaceflight can also affect gut microbial and immune homeostasis.[33] Gut microbiota is a crucial regulator of amino acid metabolism and the intestinal amino acid pool.[35] Germ-free mice studies provide evidence for this relationship, showing host gut microbiota distribution affecting the amino acid profile of the host GI tract, demonstrating a clear dependence of amino acid metabolism on the gut microbiota.[36] Similarly, *Escherichia coli* relies on numerous amino acids for microbial protein synthesis.[37] When *in vitro* models were used during spaceflight, a potent increase in *E. coli* growth occurred compared with ground-based models.[30,34] However, human models have not examined the effects of spaceflight on *E. coli* composition. In addition, Zhang *et al*.[28] reported an upregulation of protein abundance in *E. coli in vitro* that were actively involved in arginine and proline metabolism, several of which served as transporter subunits for lysine, ornithine, and arginine along with glutamate and aspartate. Spaceflight has even been shown to cause an increase in *Bacteroidetes* and a decrease in *Firmicutes* in female mice after 13 days.[38] Human gut microbiota are dominated by five bacterial phyla: *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria*, and *Verrucomicrobia* with *Bacteroidetes* and *Firmicutes* making up about 80–90% of the species in adult human gut.[39,40] Increases in *Bacteroidetes* seen in female mice after spaceflight are similar to responses reported for diet-induced weight losses in humans and mice.[40–43] This shift

Table 2. Effects of microgravity on concentrations of amino acids in plasma, urine, liver, and immunocytes.

Model	Intervention	Sample	Significant findings	Reference
Human				
Identical twins (2 people)	Twin #1 – Earthbound Twin #2 – 340 days on ISS	PBMCs Plasma Urine	• Many altered AA metabolites • Increased plasma and urine 5-oxoproline concentration	Garret-Bakelman *et al*.[27]
Animal				
Sprague-Dawley rats (21 rats)	8 days on STS-63, ground module, or Vivarium	Liver	• 33% decreased total liver glutathione post-flight • Reduced hepatic glutamate concentration post-flight	Hollander *et al*.[51]
C57BL/6J male mice (18 mice)	30 days on ISS with or without artificial gravity, or ground controls	Liver	• Reduced sulfur-containing AA (e.g. taurine, cysteine), their metabolites, histidine, and glutathione post-flight	Kurosawa, *et al*.[52]
Cell				
Macrophage (human)	325 s vs. 11 days microgravity exposure	Primary human macrophage	• Short-term: increased AA concentrations • Long-term: decreased AA concentrations	Thiel *et al*.[49]
Macrophage (human)	11 days on ISS vs. Ground controls	Primary human M1 macrophage	• Reduced ICAM-1 expression post-flight	Tauber *et al*.[54]
Macrophage (mouse)	Simulated microgravity vs. 1×*g* controls	Primary mouse macrophage	• Over-expressed C/EBPβ, up-regulated IL-6, downregulated IL-12β vs. control • Greater arginase I expression vs. control	Wang *et al*.[50]
Hematopoietic progenitor cells (mouse; HPCs)	12 days on Tianzhou-1/SJ-10, simulated microgravity, or normal gravity (controls)	Mouse hematopoietic progenitor cells	• Macrophage differentiation from HPCs is inhibited vs. controls • Impaired M1/M2 polarization in simulated microgravity • Macrophage differentiation in microgravity involves p53 and RAS/ERK/NFκB pathways • Downregulated arginine, proline, glycine, serine, and threonine metabolism in flight • Downregulated alanine, glutamate, and aspartate in flight, upregulated in simulated microgravity • Greater BCAA degradation in flight	Shi *et al*.[53]

AA, amino acid; BCAA, branched-chain amino acid; C/EBPβ, CCAAT/enhancer-binding protein β; ERK, extracellular signal-regulated kinase; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; ISS, international space station; NFκB, nuclear factor kappa B; PBMCs, peripheral blood mononuclear cells; RAS, rat sarcoma; STS-63, the second mission of the US/Russian Shuttle-Mir Program, which carried out the first rendezvous of the American Space Shuttle with Russia's space station Mir.

in phyla could affect short-chain fatty acid production and possibly modulate hunger, mood, immunity, and other physiological responses.[44]

To date, the direct effects of microgravity on amino acid digestion and degradation in the human gut have not been investigated. As such, the possibility remains that the digestion of protein and the degradation of amino acids are altered during microgravity exposure. There exists a need for research in this area considering that amino acids such as glutamate, glutamine, proline, citrulline, and arginine incur a multitude of health and functionality benefits on various systems of the organism, especially the gastrointestinal tract. Of particular interest would be studies looking at the effects of microgravity on citrulline synthesis in intestinal epithelial cells, as citrulline is the major precursor to arginine.[3]

Amino acid metabolism in immune cells

Astronauts are exposed to significant quantities of ionizing radiation during spaceflight, regardless of duration. Ionizing radiation triggers the production of reactive oxygen species (ROS), which exert negative effects on cell signaling, protein metabolism, redox regulation, apoptosis, necrosis, and immune cell function.[45–47] Available evidence shows effects of microgravity on amino acid metabolism in immune cells,[48] and such results are summarized in Table 2.[9,27,49–54] Cells of the immune system are integral sites for amino acid metabolism, with specific cells (i.e. macrophages) being dependent on certain amino acids as energetic substrates (i.e. glutamine, glutamate, and aspartate).[3,55] The citrulline–arginine cycle in macrophages was discovered in 1992, whereby arginine is oxidized to citrulline and NO, with citrulline then being recycled into arginine at the expense of aspartate and ATP.[3] Arginine can also be degraded to NO at various other sites in organisms (i.e. endothelial cells, and kidneys) as NO is a gaseous signaling molecule that serves many roles in the body (i.e., the regulation of platelet adhesion and blood flow).[3] Polyamine synthesis from arginine occurs in many cell types; however, arginine can be converted to ornithine in the mitochondria of extrahepatic cells by arginase-II.[56] Polyamines are essential for the synthesis of deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), and protein in all cells.[57]

Both short-term and long-term microgravity conditions can affect immune cell amino acid metabolism. In particular, Thiel *et al*.[49] found that the concentrations of glycine, methionine, phenylalanine, arginine, threonine, cysteine, and the BCAAs in the supernatant composition of human macrophages were increased after spaceflight exposure. An increased abundance of amino acids in the culture medium could suggest an increase in protein degradation, as microgravity potentiates catabolism possibly through ROS-mediated mechanisms (e.g. the production of ROS that is highly sensitive to gravitational changes and usually occurs within seconds of microgravity exposure).[49,58] However, most consistently it has been found that ROS production and release from NR8383 macrophages decreases during short-term simulated microgravity and parabolic flight

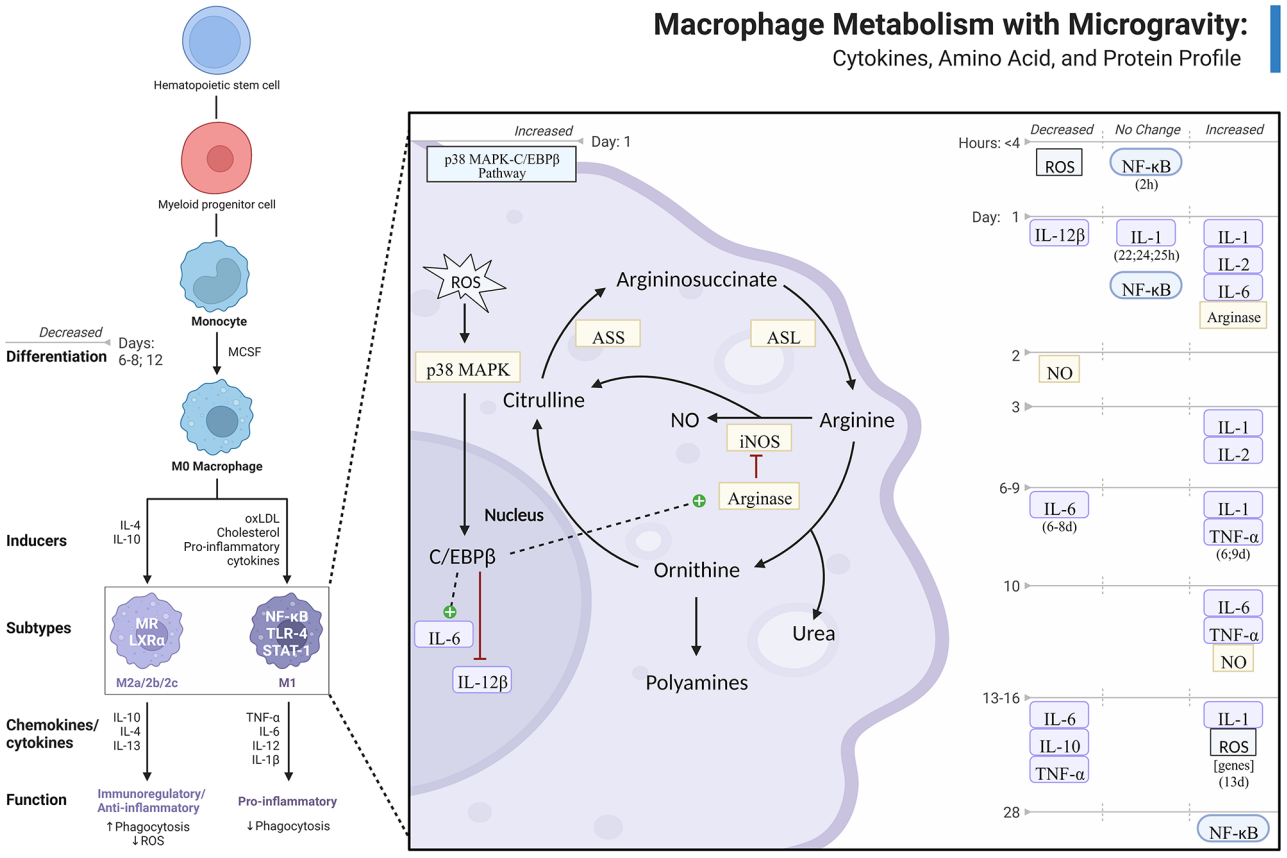

Figure 2. Macrophage metabolism under conditions of microgravity: Cytokines, amino acid, and protein profiles.
Microgravity impacts macrophage development and metabolism differently over time, and so this figure shows changes at different times, depicting how various processes, cytokines, and amino acids are related as well as to macrophage differentiation; time points (right side) are further divided, showing which markers decreased, did not change, and/or increased. A relationship is highlighted in the following: Impaired differentiation of hematopoietic progenitor cells into macrophage subtypes alters their number and function, chemokines/cytokines released, and inflammatory/immunoregulatory processes. More specific to the cell, the citrulline-arginine cycle, reactive oxygen species (ROS) production affects p38 mitogen-activated protein kinase (p38 MAPK), and in turn the expression of CCAT/enhancer-binding protein beta (C/EBPβ), influencing chemokine/cytokine secretion and upregulating arginase, which decreases the production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS). *Id est*, greater ROS may reduce NO availability, increase ornithine, and polyamine synthesis, along with altering macrophage functionality due to irregular cytokine release; or the opposite, if ROS decreases. IL: interleukin; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α: tumor necrosis factor alpha.

with upregulations in ROS-regulating genes after microgravity exposure, a phenomenon that was also observed in female mice.[58,59] Macrophage metabolism is also affected by microgravity with reductions in respiratory burst and phagocytic ability occurring after instant exposure, although changes are reversible after quick adaptation.[58,60] Amino acid metabolites have even been found in increasing concentrations as a response to microgravity. For example, a significant increase in the medium concentration of ketoleucine (α-ketoisocaproate, KIC) compared with control cells was found during short- and long-term microgravity in primary human macrophages; yet, this may not reflect *in vivo* responses given that the released KIC could be transported to the liver for catabolism.[49] Because KIC is a product of leucine transamination and can be further decarboxylated to isovaleryl-CoA in tissues, the release of KIC from cells depends on the balance between its formation and degradation. More results from primary mouse macrophage exposure to simulated microgravity reveal altered arginine degradation through the downregulation of inducible NO synthase (iNOS) by arginase-I.[50] Upregulation of arginase-I promotes the conversion of arginine to ornithine and eventually polyamines, yet decreases NO production in macrophages, which has been shown in three-dimensional-cultured macrophages during 2 days of simulated microgravity.[61] However, NO synthesis and secretion from macrophages may be time dependent as longer duration spaceflight (10 days) in male rats yielded enhanced NO secretion.[62] Direct comparisons of studies assessing macrophage responsiveness to microgravity are difficult to make due to the wide variety of methodologies used in the research. Inconsistent findings may result from differences in either microgravity duration, types of experiments (*in vivo*, *in vitro*, human, and mouse, etc.), or whether actual or simulated microgravity was used.[63] Figure 2 depicts results from several studies[50,53,54,64,65] as a synthesized drawing of macrophage amino acid and protein profile and how microgravity can affect metabolism and cytokine output. Dysregulated cytokine output could stem from altered macrophage metabolism in microgravity. A wide range of results indicate that various interleukins (IL-1, 6, 10, and 12) and tumor necrosis factor (TNF) are gravisensitive and may depend on exposure duration.[44,48,59,61,62,66–68]

The National Aeronautics and Space Administration (NASA) Twin study is one of the most comprehensive studies to date on the effects of spaceflight on human physiology.[27] Amino acids were reported to be some of the

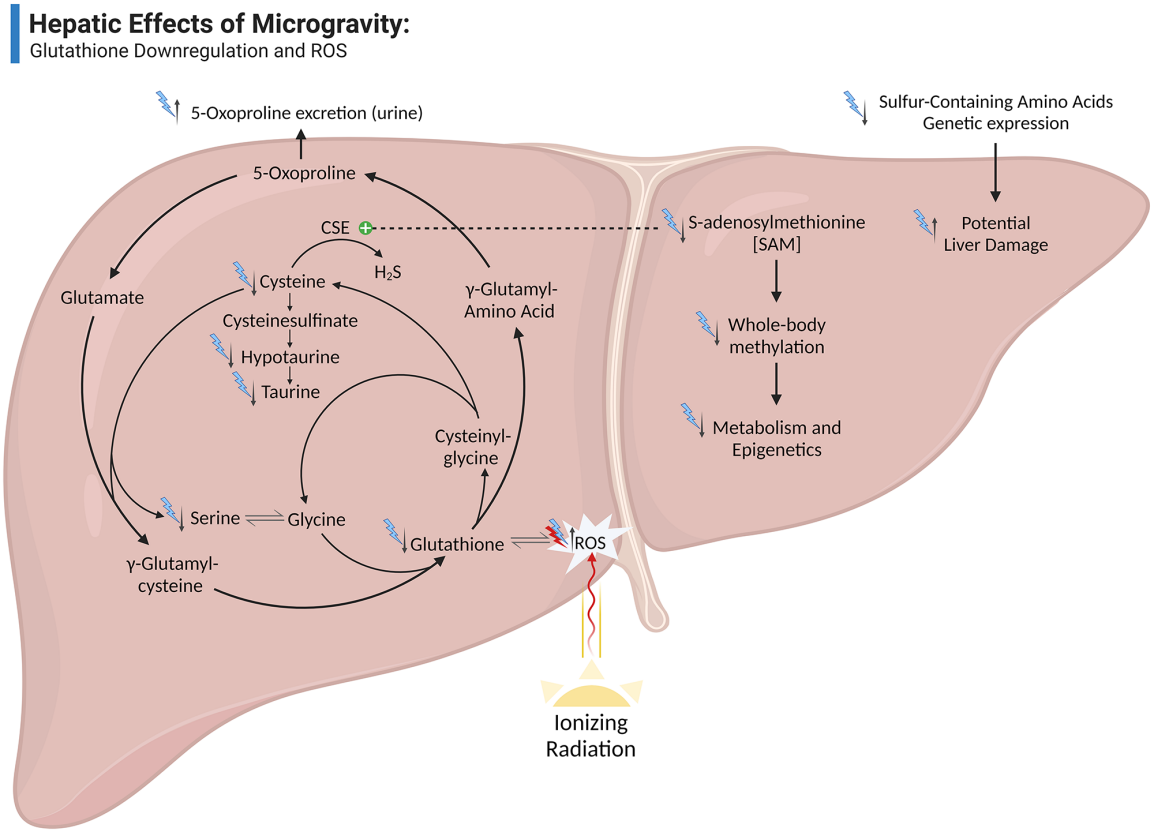

Figure 3. Hepatic effects of microgravity: Reduced glutathione synthesis and enhanced production of reactive oxygen species.
A comprehensive view of how and where exposure to ionizing radiation (red bolt) and microgravity (blue bolt) affect hepatic protein metabolism directly and indirectly, by stimulating reactive oxygen species (ROS) as well as downregulating glutathione (GSH) synthesis, respectively, leading to inhibited protein synthesis and increased breakdown. The downregulation of sulfur-containing amino acids, S-adenosylmethionine (SAM), and other intermediary compounds has many potential downstream effects, including oxidative stress regulation (e.g. decreased hydrogen sulfide (H_2S) production), liver health, metabolism, and epigenetics. CSE: cystathione-γ-lyase.

most significantly altered metabolites out of the myriad of variables collected. Significant alterations in amino acid metabolites in urine and plasma were seen after long-term exposure to spaceflight. Of note, 5-oxoproline concentration was increased in urine and plasma during spaceflight.[27] 5-oxoproline is an intermediate in the γ-glutamyl cycle (glutathione synthesis), which relies on adequate glycine, glutamate and cysteine concentrations.[69] Increased 5-oxoproline in urine and plasma could indicate dysregulated glutathione synthesis in tissues (primarily the liver), as was seen in rats exposed to microgravity for 8 days.[51] Conversely, Rizzo *et al*.[70] reported an increase in glutathione concentration in red blood cells of mice in response to oxidative stress. Glutathione (a major antioxidant in mammalian cells) is mainly synthesized in the liver, and alterations to hepatic glutathione concentrations can result in profound downregulations in antioxidative capacity.[71] Thus, a reduction of glutathione synthesis can exacerbate the onset of oxidative stress in astronauts. Likewise, sulfur-containing amino acids (cysteine and taurine) and their intermediates (serine) have shown to be downregulated in the liver of rats after microgravity exposure, which induced glutathione downregulation and exacerbated liver damage in the presence of ROS.[52,63] Another integral function of glutathione is that it exerts control on vitamin C metabolism, meaning alterations in glutathione synthesis would have direct implications on vitamin C status, ultimately affecting cellular functions in the innate and adaptive immune systems.[16,72,73] A reduction in antioxidative capacity during spaceflight supports astronaut nutrition researchers developing antioxidant cocktails rich in various antioxidants including vitamin C.[46,74]

Several amino acids that serve as precursors to antioxidants or as antioxidants themselves are significantly impacted by microgravity. Chen *et al*.[9] examined metabolites in the urine of humans before, during, and after 45 days of head-down tilt bed rest. Dynamic changes were observed for taurine, glycine, betaine, creatine, and glutamine,[9] reflecting alterations in glutathione synthesis, antioxidative capacity mediated by taurine in liver and skeletal muscle, reduced oxidant-protective capacity of creatine, and glutamine-induced whole-body pH regulation, respectively. Taurine also offers cardioprotective effects specifically by downregulating angiotensin II and angiotensin-converting enzymes (ACE) found in the lungs and kidneys, potentially contributing to the hypovolemic response seen in microgravity.[9] ACE converts angiotensin I to angiotensin II, thereby increasing blood pressure and inhibiting the vasodilatory kinin-kalikrein system.[75–77] Taurine promotes vasodilation by downregulating ACE, sparing kinins to promote inflammation and vasodilation.[75–77] Taurine is the most abundant beta amino acid in the skeletal muscle, heart, brain, and eyes across various species, so an increased urinary concentration of taurine in microgravity could be indicative of oxidative damage in these tissues.[3] Figure 3 shows the reduced synthesis of glutathione from cysteine, and the

Table 3. Effects of microgravity on amino acid metabolism in the musculoskeletal system.

Model	Intervention	Sample	Significant findings	Reference
Human				
Male volunteers (7 people)	45-day head-down tilt bed rest (HDBR)	Urine	• Increased urinary excretion of metabolites and AAs linked to cardiovascular issues and deconditioning of muscle, and bone • Urinary excretion of glutamine, glycine, guanidinoacetate, and creatinine synonymous with the amount of muscle lost	Chen *et al*.[9]
Males + Females (20 people)	21 days, 6° HDBR	Gingival crevicular fluid	• Increased expression of MMP-8 and MMP-9	Rai *et al*.[31]
Males + Females (13 people)	28-day bed rest with or without AA supplement	Urine	• Greater concentration of urinary deoxypyridinoline in AA group	Zwart *et al*.[81]
Crew members (7 people)	9.5 days on SLS shuttle	Urine	• Reduced nitrogen balance post-flight • Increased muscle protein synthesis on day 8 of flight	Stein *et al*.[83]
Astronauts (14 people)	9.5- or 15-day spaceflight	Urine Plasma Saliva	• Day 1 caloric intake dropped compared to preflight values • Nitrogen retention and balance decreased in early stages of flight • Loss of lean body mass ≈1 kg	Stein *et al*.[84]
Payload crew members (4 people)	15 days on SLS-2 shuttle	Plasma	• Altered plasma EAAs and BCAAs concentrations • Increased plasma leucine and isoleucine concentrations	Stein and Schluter[85]
Astronauts (2 people)	Female [F]: 14.7-day flight Male [M]: 8.9-day flight	Urine Plasma	• Elevated bone resorption markers; urine pyridinolines; plasma TRACP • BMD decreased ≈3% in lumbar spine; increased in skull post-flight for both subjects • Leg muscle PCSA decreased 10-15% post-flight, > 1 month recovery	Miyamoto *et al*.[86]
Male astronauts (3 people)	28-day flight with exercise countermeasures	Urine	• Increased urinary hydroxyproline concentration nitrogen excretion	Whedon *et al*.[87]
Males (8 people)	72 h unilateral lower limb suspension	Vastus lateralis interstitial fluid	• Increased concentration of 3-methylhistidine in skeletal muscle	Tesch *et al*.[90]
Animal				
Neonatal rats	16 days on STS-90 Hindlimb suspension	Gastrocnemius	• Increased mRNA expression of cathepsin and protein ubiquitination	Ikemoto *et al*.[89]
Cell				
Myoblasts	36 h 3D clinostat	C2C12 Myoblasts	• Increased FOXO1 activation and protein ubiquitination	Baek *et al*.[88]

AA, amino acid; BCAAs, branched-chain amino acids; BMD, bone mineral density; EAAs, nutritionally essential amino acids; C2C12, C2C12 is a myoblast cell line; FOXO1, Forkhead box class O-member protein 1; MMP, matrix metalloproteinase; PCSA, physiological cross-sectional area; SLS, space launch system; STS-90, a 1998 Space Shuttle mission flown by the Space Shuttle Columbia; TRACP, tartrate-resistant acid phosphatase (a marker of bone resorption).

production of taurine, as well as the adverse effects of ROS, exacerbated by ionizing radiation exposure on endogenous antioxidant capacity.[9,27,45,52,63]

Amino acid metabolism in skeletal muscle

One of the most heavily reported complications of spaceflight or microgravity analogs is the loss of body protein, particularly lean body mass protein.[2,78–81] Refer to Hackney and English[82] for a comprehensive summary on the effects of gravitational unloading on skeletal muscle and bone. Gravitational unloading removes physical stress on the musculoskeletal system, leading to exaggerated lean mass and bone loss.[81] Table 3 reviews study outcomes of microgravity/spaceflight on amino acids, their metabolites in skeletal muscle and bone, and molecules affecting protein degradation and amino acid profiles.[9,31,81,83–90] Studies have found significant reductions in intramuscular protein turnover caused by dysregulated protein synthesis and increased protein degradation.[2,83,91] Initial exposure to microgravity has been shown to elicit an increase in muscle protein synthesis;[84,92] however, after 8 days of spaceflight a net decrease in muscle and whole-body protein synthesis occurs.[2] Typically, an increase in plasma BCAAs (leucine, valine, and isoleucine), a hallmark of net protein degradation, occurs in initial responses to spaceflight with a concomitant rise in urinary cortisol levels.[84,85] Cortisol is a mediator in skeletal muscle catabolism by acting on muscle to release gluconeogenic substrates, thus possibly leading to a net release of amino acids from skeletal muscle, resulting in net muscle protein breakdown.[93,94] A loss of muscle nitrogen has also been reported during spaceflight in humans, which yields a negative nitrogen balance.[84]

Another possible explanation for loss of skeletal muscle during spaceflight is an altered anabolic hormone profile. Older studies in men have shown a marked reduction in serum testosterone (50%) during short-term spaceflight.[2,95] Specifically, the ratio of anabolic to catabolic hormones is shifted, with serum concentrations of cortisol and testosterone rising and decreasing, respectively.[2,95] However, results of more recent research indicated that the concentrations total, free, and bioavailable testosterone in the serum of humans were not altered during long-duration spaceflight and were reduced only on the day of landing after either short- and long-duration spaceflight.[96] Smith *et al*.[96] suggested that the discrepancy in serum testosterone could be due to inadequate caloric intake by astronauts during the earlier spaceflight.[2,95] Nonetheless, the ratio of cortisol and testosterone concentrations was perturbed during spaceflight and on the day of landing.[96] The rise in cortisol, whether from physical inactivity or microgravity, can increase glutamine, alanine, and phenylalanine efflux from skeletal muscle.[97] In fact, bed rest models show loss of intramuscular glutamine

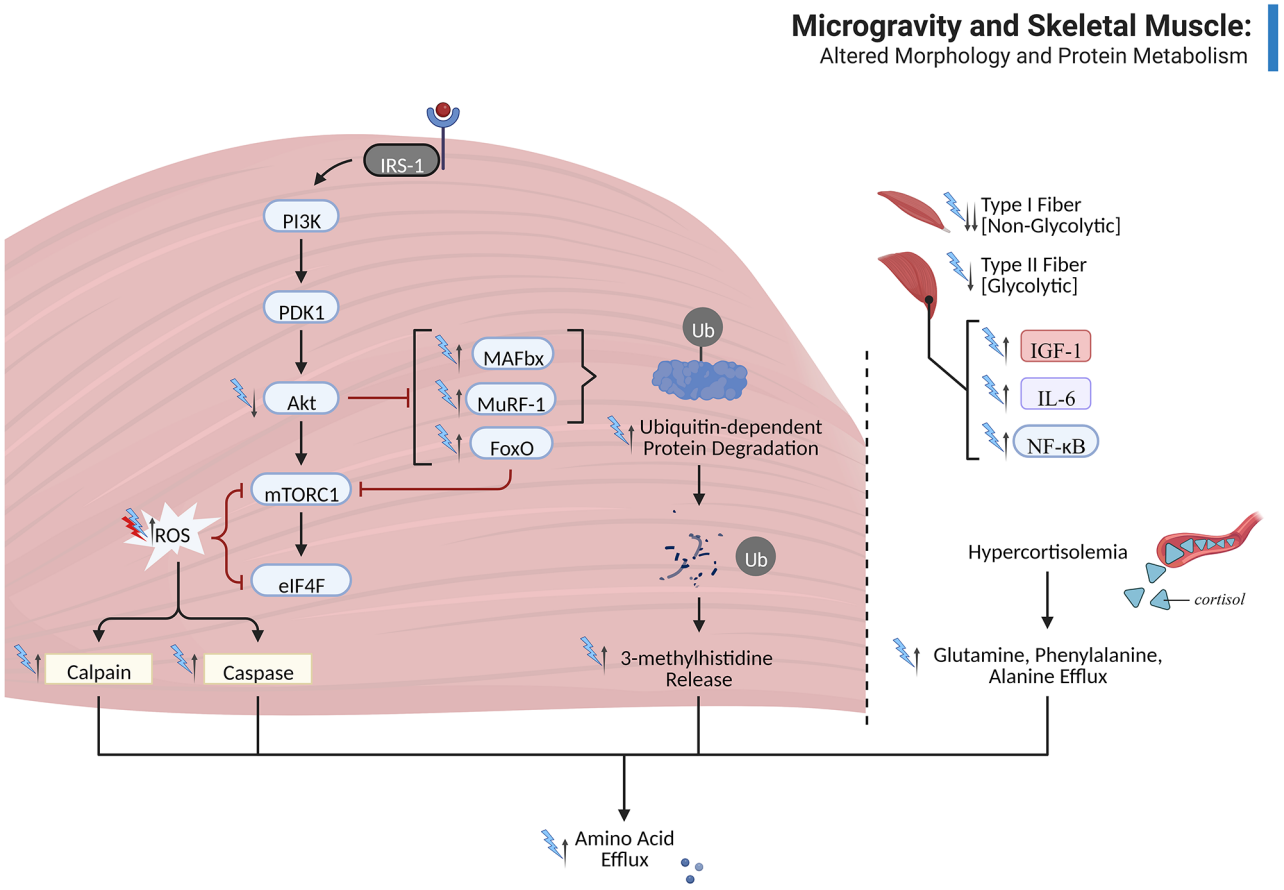

Figure 4. Microgravity and skeletal muscle: Altered morphology and protein metabolism.
This graphic depicts how muscle protein metabolism may be altered by microgravity, with increased protein breakdown, leading to atrophy and subsequent amino acid efflux, including elevated urinary 3-methylhistidine concentration. Typically altered muscle fiber phenotype ratio is also seen, with slow-twitch (type I) less present than fast-twitch (type II) fibers, due to the cytoprotective role that insulin-like growth factor-1 (IGF-1), interleukin-6 (IL-6), and NF-κB play in preserving the type II myosin heavy chain phenotype, whereas type I myosin ATPase shifts toward type II and type I motor units degrade. Moreover, there is a reduced ability of the insulin receptor substrate-1/phosphoinositide-3 kinase/protein kinase B (IRS-1/PI3K/Akt) signaling pathway to inhibit ubiquitin ligases (Muscle RING-finger-1 (MuRF-1) protein, Atrogin-1/Muscle atrophy F-box (MAFbx)), and the Forkhead box protein O (FoxO) transcription factor, owing to decreased Akt, thereby inhibiting the phosphorylation of mechanistic target of rapamycin complex 1 (mTORC1) and eukaryotic initiation factor 4F (eIF4F). ROS accumulation inhibits mTORC1 and eIF4F further while also activating proteolytic enzymes (e.g., calpains, caspases). Concurrently, an increase in circulating cortisol concentrations promotes the catabolism of glutamine, phenylalanine, and alanine for ATP provision.

concentrations as a result of hypercortisolemia combined with physical inactivity, which highlights the need for astronauts to partake in daily aerobic and resistance exercise along with maintaining proper nutrient intake.[97] Moreover, decreased intramuscular glutamine concentrations might influence skeletal muscle protein breakdown,[98] a metabolic relationship that is illustrated in Figure 4. By contrast, exogenous testosterone treatment during long-duration bed rest can result in an increase or no change in lean body mass in conjunction with or without physical exercise, respectively.[99]

Spaceflight is associated with a dramatic increase in muscle protein degradation.[100] Microgravity and its analogs have shown increased excretion of biomarkers for protein breakdown. For instance, Tesch *et al*.[90] found an increase in urinary 3-methylhistidine after 72 h of unloading. 3-methylhistidine can be generated when histidine residues in muscular actin and myosin become methylated after the synthesis of these proteins. This amino acid metabolite is excreted through urine in higher amounts during catabolic states, suggesting an increase in muscle protein breakdown.[101] Stein *et al*.[84] offered supporting evidence that an increase in urinary 3-methylhistidine is seen shortly after spaceflight. In addition to altered metabolic profile, the morphology and composition of skeletal muscle is significantly affected. Microgravity primarily affects type I, slow-twitch oxidative muscle fibers, resulting in a shift from slow to fast myosin heavy chain expression.[102] Shifting from slow to fast-twitch phenotype causes an initial rise in type II, glycolytic composition with some data showing a possible protective role of signaling factors such as insulin-like growth factor-1, cytokines such as interluekin-6, and stress-related genes on type II muscle.[102] Concomitant with the enahnced concentrations of markers for muscle protein breakdown and the shift of fiber types is an increase in the expression of ubiuitin-proteosome ligases that induce protein degradation.[100] mRNA levels for ubiquitin ligases, Atrogin-1/Muscle atrophy F-box (MAFbx), and muscle RING-finger-1 (MuRF-1), along with the Forkhead box class O-family protein transcription factor, were increased in rats during hindlimb suspension, thus stimulating muscle protein degradation and lending to decreases in the muscle cross-sectional area in slow- and fast-twitch type muscle fibers.[102,103] The overall effects of microgravity exposure on skeletal muscle morphology and protein metabolism are depicted in Figure 4, based on results from

the studies discussed in this section, including evidence for increased secretion of inflammatory markers and enhanced ROS production causing an increase in calpain and caspase activity.[100,102–104]

Concomitant with significant skeletal muscle loss, astronauts experience an increase in bone resorption. Studies have consistently found a substantial loss of bone mineral from the skeleton during spaceflight.[82,86,87] These same studies reported an increase in urinary calcium and hydroxyproline. Hydroxyproline, found in collagen, is created from proline as a post-translational process and is subsequently released as collagen degradation accompanies bone resorption.[105] One study of amino acid supplementation in humans intended to mitigate bone loss during simulated weightlessness found increased levels of calcium in the urine along with decreased bone mineral content and decreased urinary pH.[81] Authors attributed these effects to possible loading of sulfur-containing amino acids, specifically methionine, that were incorporated in the amino acid supplement provided. The sulfur from these amino acids could have contributed to mild metabolic acidosis, which increases bone resorption.[81] These results highlight the necessity for a proper balance of amino acid consumption.

It should be noted that the cardiovascular system undergoes adaptations in microgravity.[12] Cardiovascular deconditioning is a hallmark adaptation during spaceflight with astronauts experiencing hypovolemia, altered peripheral resistance, changes in central venous pressure, and disturbed baroreflex response, leading to orthostatic intolerance once they become earthbound upon landing.[106–109] Readers are referred to Vernice *et al*.[110] and Jirak *et al*.[111] on the effects of long-duration spaceflight and microgravity on cardiovascular health. To date, little is known about cardiovascular amino acid metabolism in microgravity. Some cell culture studies suggested an onset of endothelial dysfunction that was related to the altered expression of endothelial and inducible NO synthases.[112–115] Endothelial cells are highly gravisensitive cell types that, in response to altered gravity states, undergo various morphological, functional, phenotypic, and behavioral changes.[112–115] Future studies are warranted to determine whether amino acid metabolism in the cardiovascular system is altered in astronauts or in animals under the conditions of microgravity.

Importance of dietary amino acid intake

Amino acid deficiencies can exert profound effects on skeletal muscle, immune system, and the radiation-protective response in astronauts. It is essential that astronauts meet the recommended dietary allowances for amino acids; however, some amino acids are overlooked in protein consumption guidelines. For example, arginine is considered by many to be a conditionally essential amino acid; yet, during spaceflight (in a catabolic state) arginine consumption becomes increasingly important not only for NO synthesis but for protein and creatine synthesis and cardioprotective effects.[1] It has been reported that decreased food intake is a common occurrence among astronauts during spaceflight,[116,117] with numerous causative factors such as a lack of time, radiation exposure, psycho/social factors, and/or poor palatability of processed foods on space vehicles.[118,119] However, in recent years, adequate energy intake during spaceflight has received much attention.[1,96] A greater amount of dietary proteins will be degraded to amino acids when energy needs are not met as compared with sufficient energy consumption. Note that dietary glutamate, glutamine, and aspartate are the major sources of energy for the small intestine of mammals,[3] possibly including humans. Alanine will be transported out of skeletal muscle and into the liver for gluconeogenesis to help produce glucose in response to a reduction in food intake.[120] Loss of muscle mass and strength, depressed immune response, kidney stone formation, increased bone resorption, and a decreased pH (more acidic) in the blood due to increased ketone body formation are all potential consequences of astronauts not meeting energy needs.[121–125] Thus, it is not only imperative that astronauts consume adequate amounts of not only amino acids, but also carbohydrates, fats, minerals, vitamins, and fluids.[1]

Maintaining proper protein intake is paramount to negate a plethora of adverse effects, like those mentioned above, induced by microgravity and radiation. Some major issues associated with amino acid nutrition and astronauts include negative nitrogen balance, glutathione deficiency and dysregulated metabolism of antioxidative amino acids leading to oxidative stress, and altered blood flow caused by the cephalic shift of fluids as a result of microgravity and alterations in NO synthesis and bioavailability.[1,2,9,51,126] Decreased NO bioavailability, due to decreased synthesis from arginine as a result of a perturbed citrulline-arginine cycle, could exacerbate the cephalic shift of fluids. Increasing amino acid consumption can act to mitigate the maladaptive processes occurring in astronauts during space travel. During spaceflight, protein intake ranging from 1.3 to 1.6 grams per kilogram of body weight daily for astronauts undergoing exercise countermeasures is recommended.[82,117,127,128] Although nutritional practices advocate for the food matrix and proteins from whole foods, supplementation with amino acids or other protein sources should not be overlooked. Other factors concerning delivery cost to the Space Station, efficacy, and the relationship between bone loss and the intakes of sulfuric amino acids are also being discussed in regards to protein supplementation.[81,82,129] Nonetheless, supplementation with amino acids serving functional roles in the body (i.e. EAAs and some AASAs such as arginine, glycine, glutamine, glutamate, aspartate, and proline) can act to promote skeletal muscle protein synthesis, inhibit muscle protein breakdown, and increase the synthesis of bioactive molecules.[3] These effects would likely be enhanced with the undertaking of a resistance and cardiovascular exercise countermeasure training program. Therefore, studies assessing the effects of functional amino acids in musculoskeletal health in microgravity are warranted.

Adequate consumption of the proteinogenic amino acids, including the AASAs (e.g. arginine, glycine, glutamine, glutamate, aspartate, and proline), should be stressed.[3] Along with arginine, glutamine and BCAAs can also stimulate muscle protein synthesis with glutamine offering pleiotropic effects by controlling ammonia concentration and blood pH.[130–132] Leucine has been extensively studied in nutrition and exercise, consistently showing to be a

primary driver of muscle protein synthesis around the times of exercise training or in catabolic states.[133,134] In addition, studies with sarcopenic older adults and cancer patients assessing whey protein consumption with adequate levels of leucine (3-6 grams) have reported improvements in not only skeletal muscle outcomes, but also immune function by increasing natural killer cell function and IL-12 concentration.[135–137] Leucine also produces an anti-catabolic metabolite, β-hydroxy-β methylbutyrate (HMB), which has been implicated in blunting muscle protein degradation in states of muscular disuse or atrophy-inducing conditions, making it a potential target of supplementation in astronauts.[138,139] However, no research has been done to determine whether HMB supplementation may affect skeletal muscle protein metabolism in microgravity. Consumption of some non-proteinogenic amino acids such as taurine and β-alanine are also recommended.[3] β-alanine serves as the rate-limiting molecule in carnosine synthesis.[140] Carnosine is a primary intramuscular pH buffer, and β-alanine supplementation has been shown to enhance carnosine levels in the muscle while providing ergogenic effects during exercise lasting one to four minutes.[140] As previously discussed, taurine offers a cytoprotective role by acting as an antioxidant and can also elicit indirect cardioprotective effects.[9] Although these amino acids provide health-conferring benefits, some caution should be taken when consuming taurine and β-alanine as they compete for the same transporter (Tau-T) into skeletal muscle.[140,141] Theoretically, overconsumption of one can lead to an inhibited uptake of the other, which has been seen in animal models, but not in humans.[141,142] Studies that assess the relationship between taurine and β-alanine during spaceflight and the implications it may have on health outcomes are encouraged. Improving astronauts' antioxidative capacity is of high importance. Exposure to ionizing radiation perturbs redox pathways and can lead to higher production of ROS.[45] Thus, consuming not only amino acids that prelude antioxidants or act as antioxidants themselves, but vitamins and minerals in adequate amounts will provide a protective effect against the cytotoxic and degradative effects of ROS on immune function, protein metabolism, and multi-organ damage. Adequate consumption of glycine can augment glutathione synthesis, thereby improving the antioxidative response in microgravity.[143] N-acetylcysteine supplementation has also been shown to enhance glutathione status in individuals and can be seen as another potential supplementation protocol for astronauts.[144] Glutamine and glutamate have the potential to alter enterocyte proliferation and function while strengthening macrophage activity, which is vital as the gut houses a majority of the immune system.[145,146] In addition, creatine is one of the most widely studied metabolites of amino acids and highly effective ergogenic compounds available in the body.[147] The studies on the effects of creatine have traditionally been examined in regards to skeletal muscle performance and outcomes; however, in more recent times, the effects of creatine on immune response have been explored. Studies suggest creatine consumption influences the immune response and can serve as a benefit to the user; granted, overconsumption can lead to negative health outcomes.[148] No studies have yet been conducted on glycine, glutamate, glutamine, and creatine as potential immunoprotective compounds in microgravity.

Finally, anti-muscle wasting drugs may be used in conjunction with amino acid supplementation to mitigate the marked reductions in skeletal muscle mass during spaceflight. In this regard, it is noteworthy that Lee *et al*.[149] recently reported that the administration of a soluble form of the activin type IIB receptor (which binds to both myostatin and activin A (inhibitors of muscle growth)) to adult mice could ameliorate skeletal muscle and bone losses during a 33-day period of spaceflight. It remains to be determined whether a combination of this drug with functional amino acids may have additional benefits on the health of individuals in microgravity.

Conclusions

Amino acids serve a wide variety of roles in the body, and their consumption can adequately serve as a nutritional countermeasure to the maladaptive responses seen in astronauts. The effects of microgravity influence a litany of cellular responses in which amino acids are involved, causing astronauts to experience altered gut microbial composition, immune dysregulation, skeletal muscle atrophy, oxidative stress, hypovolemia, and bone loss. Maintaining adequate protein consumption (1.3–1.6 g/kg/bodyweight/day) with proteinogenic amino acids, AASAs, and some non-proteinogenic amino acids are emphasized as astronauts venture into microgravity. Consumption of adequate amounts of amino acids can stimulate metabolic pathways involved in muscle protein synthesis, antioxidant responses, and promote the upregulation of bioactive molecules needed for survival. Studies involving amino acid consumption during microgravity exposure, or during earth-based microgravity analogs, are besought as the field is lacking in this area. Therefore, seminal contributions regarding amino acid consumption or supplementation relative to astronaut health and physiology will offer insight into the potential combative/therapeutic effects they may have on changes imposed by microgravity, propelling the field of spaceflight nutrition in an upward trajectory.

AUTHORS' CONTRIBUTIONS

BLD and GW conceived this writing project. BLD, RS, and GW wrote the initial draft of the manuscript. Figures were created by RS and BLD. RS, RK, and GW contributed to revisions of the article.

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