Human Physiological Limitations to Long-Term Spaceflight and Living in Space

Lawrence H. Winkler

INTRODUCTION: Despite all our dreams and enthusiasm, the essential question of whether our species can ever live permanently in space remains unanswered. The 1975 NASA Ames Design Study on Space Settlements demonstrated how human physiology constrains and determines human habitat design in space. Our scientific understanding about the risks of and standards for microgravity (and rotation rate if centrifugally generated), ionizing radiation, and atmosphere pressure and composition, remains inadequate a half century later. In addition, there are newly recognized physiological challenges to living safely in space, including spaceflight-associated neuro-ocular syndrome (SANS), extravascular hemolytic anemia, and other factors that affect every human cell and organ system. A comprehensive review was conducted to establish what we have learned and what is still required to know about the pathophysiology of long-term space travel and living in space since my first report in 1978. The results determine not only how, but if we can realistically plan to inhabit the cosmos that surrounds us.

KEYWORDS: Stanford Torus, space medicine, life support.

Winkler LH. *Human physiological limitations to long-term spaceflight and living in space*. Aerosp Med Hum Perform. 2023; 94(6):444–456.

In the summer of 1975, a small group of engineers, scientists, and students participated in a NASA program to design a space settlement for 10,000 inhabitants sited at L5.[39] The technical director of the study, Princeton professor Gerald K. O'Neill, envisioned the colony as a cylinder with an interior "like the French countryside."[71]

What O'Neill failed to appreciate was the magnitude of control exerted on spacecraft design by safe physiological criteria.[32] Our group took great care to create an environment that adequately protected humans from the space hazards known about at the time (see **Table I**). The Stanford Torus evolved out of conservative necessity in the absence of more permissive space-based in vivo data.

As a life support consultant for the 1975 study,[103] I evaluated what we have learned about the physiological hazards of spaceflight[2,33,55] and the interval advances that may have made it safer.[69,109] This paper will review the hazards of long-term spaceflight and living in space, as well as the pathophysiological consequences and potential countermeasures. It will conclude with a narrative of what we have learned after almost half a century of innovation.

HAZARDS TO LONG-TERM SPACEFLIGHT AND LIVING IN SPACE (LTS/LIS)

Space is a physical environment inherently hostile to human habitation.[7] With an average proton density of 5.9 protons/m^3 and an average atomic density between galaxies of less than 1 atom/m^3, space is an imperfect vacuum of almost nothing, punctuated by plasma, orbital debris, and micrometeoroids. The extreme temperatures of space range from −272.15°C in the Boomerang Nebula in the constellation Centaurus, to 15,000,000°C at our sun's core (and hotter). However, with the right mix of spacecraft interior gas composition and temperature regulation, life support design can compensate for these first two hazards.

From the University of British Columbia, Vancouver, British Columbia, Canada.

This manuscript was received for review in October 2022. It was accepted for publication in February 2023.

Address correspondence to: Dr. Lawrence Winkler, 2343 Arbot Road, Nanaimo, British Columbia, Canada V9R 6S9; drwink@shaw.ca.

DOI: https://doi.org/10.3357/AMHP.6190.2023

TABLE I. Physiological Design Criteria 1975.

PHYSIOLOGICAL DESIGN CRITERIA	VALUE
Pseudogravity	0.95 ± 0.5 G
Rotation Rate	≤1 rpm
Radiation Exposure Limits	≤0.3 rem/year
Magnetic Field Intensity	0.6 ± 0.3 gauss
Atmosphere	
P_{O_2}	2.3×10^2 mb
P_{N_2}	2.7×10^2 mb
P_{CO_2}	$<4.0 \times 10^2$ mb
PH_2O	1.3×10^3 mb
Temperature	23 ± 8°C

P_{O_2} = oxygen partial pressure; P_{N_2} = nitrogen partial pressure; P_{CO_2} = carbon dioxide partial pressure; PH_2O = water partial pressure

Radiation

Each of the three main types of space radiation possesses its special hazard profile. Large flux emissions of energetic ions from the sun occur as sporadic and cyclical solar particle events (SPEs). Spacecraft shielding and EVA suits can handle protons with energies less than 30 MeV, but higher fluxes from solar flares or coronal mass ejections may penetrate shielding and exacerbate the biological effects from other exposures. Acute radiation sickness (ARS) recovery may be hindered by changes in immune status, skin burns, blood loss, and slower wound healing. In addition, solar UV radiation causes an increased incidence of skin cancer.

The major concern for deep space missions, being both isotropic and constant over time, is galactic cosmic rays (GCRs). These are high atomic number, high-energy (HZE) particles with an energy spectrum of 1–10,000 MeV.[16] Every cell nucleus in the body will be traversed by a high-energy cosmic proton every 3 d en route to Mars, each with the potential for causing complex clustered double-stranded breaks in DNA.[108] The radiation field on Mars is a hundred times more intense than on Earth.[17]

Two diffuse bands of Van Allen belt charged particles are trapped in Earth's magnetosphere. The daily pass through the South Atlantic Anomaly (SAA) accounts for 50% of the cumulative radiation received by International Space Station (ISS) astronauts.[17] Because the zone contributes no exposure to missions beyond low Earth orbit, the key to avoiding its radiation is to traverse quickly.

Gravity

On Earth, gravity is necessary for rain to fall, water to drain, heat to dissipate, and air and water to separate. In the evolution of all planetary life, gravity has been a constant factor.[67] Small variations in this weakest of the four fundamental physical forces of nature have an impact on organism health and function.[40,95]

Gravitational effects on many-celled organisms are profound. Terrestrial survival required an inner or outer skeleton to cope with buoyancy loss and increased loading. Vertebrate postural stability, structural support, mobility, fluid distribution, and circulation hydrodynamics evolved.

Species that alternate between horizontal and vertical positions require more complex systems for balance/z-vector sensing, fluid regulation, and locomotion. Humans have developed a subconscious "1 g mentality." In microgravity, nothing is pushed together, everything is pulled apart. Subtle biological changes due to altered gravity are difficult to define over a single generation. Unlike plants, no vertebrate has completed a life cycle in microgravity. Humans have spent about 1% of a life cycle in space, far fewer than the 20,000 generations of 1-g evolution that resulted in our terrestrial adaptation.

Isolation and Confinement

Perhaps the most enigmatic hazard to human long-term space travel and living in space is species generated. Several inherent elements of spaceflight confinement threaten crew productivity, health, and mission success.

Spacecraft habitability and human-machine stressors. Lack of privacy, circadian rhythm alterations from constant sterile interior or short periodic exterior lighting, makeshift sleep facilities, lack of natural UV exposure, chronic vibration/noise, increased carbon dioxide levels, housekeeping and hygiene issues, clothing uniformity, and support systems separation can lead to feelings of isolation, loss of spatial capacity, altered consciousness, and impaired coordination. Ionizing radiation can modulate psycho-emotional status and exert an anxiogenic effect.

Sensory distortion from crew displays/interfaces, intelligent machines/tool interactions, challenges of hand-eye coordination, cognition, information processing, memory and workload levels, and dangers and risks associated with physical hazards (such as space debris and equipment failure or malfunction) can contribute to mission tension.

Psychological and psychosocial stressors. Menu-fatigue, "anorexia in space," limited possibilities for rescue, high-risk work conditions, sleep disruptions, homesickness and loneliness, and motivational decline can lead to apathy, fatigue, psychosomatic disorders, anxiety, and depression.

Heightened friction, social conflict, and strained interpersonal relations between crew and/or ground stations, disruptions in family life, sexual attraction and tensions, and multicultural and multinational factors (e.g., communication language barriers, stereotyping, cultural misunderstandings, technology interfacing, religion and holidays, habitat aesthetics and work, and differences in management and leadership styles) pose potential threats to team cohesion and stability.

Stressors absent in near-Earth missions can intensify in deep space. "Earth-out-of-view phenomenon" leads to disconnectedness from family and friends. Pressures of crew-ground communication delays and dependence on local resources to generate water and fuel for the return flight home could result in withdrawal, territorial behavior, asthenia, irritability, attention/concentration difficulties, heightened perceptual sensitivities, physical weakness, sleep and appetite problems, and distress synergism from the other contributing factors.

PATHOPHYSIOLOGICAL CONSEQUENCES

The cumulative combined effects of these LTS/LIS hazards have cellular and organ system consequences.

Cellular Dysfunction

The effects of ionizing radiation on human cellular biology were well-documented at the time of the NASA Settlements Study in 1975, but there was little evidence that microgravity could also affect cell function. We are now aware that intracellular architectural structures sensing gravitational load convert and amplify mechanical inputs into downstream biochemical signaling cascades.[20]

Depending on the interaction of the cytoskeleton, cell adhesion molecules, force-sensing proteins, mechanically activated ion channels, and gene expression, mechanotransduction pathways affect the entire cellular life cycle.[9] A filamentous viscoelastic F-actin-cytoskeleton regulates cellular size, volume, shape change, force generation, adherence, proteins, cell-membrane lipid bilayers, and neural ion channels.[87,105,106] The "tensegrity" (tensional integrity) cytoskeletal equilibrium is disrupted in microgravity by the decreased expression of actin, Arp2/3, and RhoA proteins.[56] Cytoskeleton linking to the extracellular matrix (ECM) requires integrin transmembrane receptor clustering to enable focal adhesion. Microgravity reduces the formation, number, and area of focal adhesions per cell, and so decreases adherence, migration, and viability.

The microtubule organizing center (MTOC) that separates chromosomes during cell division is also gravity dependent. Human T lymphoblastoid cells flown on the Space Shuttle demonstrated shortened microtubules extending from poorly defined MTOCs with DNA condensation and increased Fas/APO-1 protein characteristic of apoptosis.[51] Furthermore, microgravity causes more fluid and less viscid membranes, decreasing current fluctuations through high voltage mechanically activated ion channels, which alters cellular metabolism. Mechanical unloading in microgravity reduces gene expression of focal adhesion proteins (FAK, DOCK1, and PTEN), as well as those involved in dopamine synthesis and hypothalamic 5-hydroxytryptamine 2A synthesis. This delayed differentiation and the changes in the cytoskeleton, nuclear morphology, and gene expression that occur in microgravity raise potential concerns about tumor growth and wound healing.

Organ System Dysfunction

Metabolism/bioenergetics. In microgravity, dramatic body fluid shifts centralize blood volumes. The increased metabolic energy required to pump blood to the skin surface to enable evaporative heat loss is further frustrated by skin sweat biofilm that impairs convective heat loss, along with blunted thirst and lower fluid intakes causing decreased perspiration and dehydration. Astronauts return to Earth hyperthermic, dehydrated, and with muscle mass loss.

Pharmacology is also different in space. The absence of gravity affects drug absorption, distribution, and metabolism.[6,21,30] Changes in ingested matter size and density, capillary pressure, splanchnic congestion, gastric pH, and diminished gastric emptying (further exacerbated by antimuscarinic drugs for space adaptation syndrome) alter bioavailability. Adjustments in gut microbiota (decreased *Bifidobacterium, Lactobacilli, Akkermansia*, and *Ruminococcus*; increased *Pseudobutyrivibrio* and *Fusicatenibacter*), epithelial transport, and intestinal transit time decrease GI absorption. Then, the volume of drug distribution is decreased by diminished plasma volume, increased fluid deficit, compartmental redistribution, cardiovascular deconditioning, and a decline in plasma albumin, tissue perfusion, and lean body mass. These factors, together with a suspected greater blood-brain barrier permeability (based on animal studies), could result in increased drug concentrations. Lipid-hydrolyzing and proteolytic enzyme activity is also reduced in spaceflight. Hepatic drug catabolism is limited by less portal blood flow velocity and first-pass metabolism, as well as decreases in CYP-450 monooxygenase activity, UGT1A1 and OCT2 transcription, and biliary secretion. Blood volume contraction causes a drop in renal perfusion, creatinine clearance, urinary excretion, drug-binding macromolecule concentration (producing elevated free drug fraction, t1/2, and AUC), and altered urinary epithelial transport carrier expression and function. Additionally, radiation can adversely affect drug stability.

Nutrition. Food will not be found during any journey into deep space. NASA's prepackaged foods have a stated shelf life of about 2 yr, but a Mars trip requires 5 yr of processed provisions.[11] The persistent catabolic state of spaceflight occurs from limited preparation time, menu fatigue, requisite exercise regimens, and loss of taste and smell that reduces palatability.

Moreover, protein supplement consumption leads to amino acid oxidation with nitrogen and sulfur release that impact kidney and bone chemistry. Vitamin D deficiency can occur from lack of solar synthesis due to radiation shielding and inadequate food sources. Other vitamins are susceptible to inactivation during food preparation, radiation exposure, and long-duration missions. Calcium is lost by decreased GI absorption, bone recruitment, and increased urinary excretion (contributing to kidney stone formation). Supplemental dietary calcium doesn't reverse the negative balance. And finally, RBC neocytolysis promotes iron tissue storage associated with Fenton reaction-induced oxidative damage.

Endocrine. Spaceflight-induced stressors (e.g., G-forces of launch and landing; weightlessness; radiation; noise; isolation and confinement; performance requirements; sleep deprivation; and insufficient nutrition) modify hormonal levels and their effects on kidneys, bone resorption, muscle loss, immunity, glycemic control, and endothelial response. Sympathetic activation results in fight-or-flight neuroendocrine stress responses. The adrenal medulla produces more catecholamines, and the hypothalamic-pituitary-adrenal axis secretes extra adrenocorticotropin and cortisol. Downregulation of the hypothalamic-pituitary-gonadal axis decreases serum testosterone transiently to levels that approximate aging male syndrome. Higher increases of plasma growth hormone,

prolactin, and catecholamine levels were noted after workload during spaceflight, as compared to preflight response. The hypothalamic-pituitary-thyroid axis generates less L-thyroxine and triiodothyronine, but the renin-angiotensin-aldosterone system increases angiotensin, aldosterone, and antidiuretic hormone. Less parathyroid hormone (PTH) is secreted by the calcium-parathyroid hormone-vitamin D axis and the insulin: glucagon axis increases serum glucose and insulin with a rise in insulin resistance.

Oxidative stress response augments reactive oxygen species (ROS), reactive nitrogen radicals, lipid peroxidation in erythrocyte membranes, erythrocyte superoxide dismutase and glutathione peroxidase, granulocyte superoxide and nitric oxide production, and urinary excretion of 8-iso-prostaglandin F (2alpha) and 8-oxo-7,8 dihydro-2 deoxyguanosine, with elevated markers of MDA (membrane damage), nitrotyrosine (protein damage), and 8-OHdG (DNA damage). Plasma and leukocyte lipophilic antioxidant levels, as well as concentrations of serum and salivary vitamin C and E, glutathione, and melatonin, are reduced.

Genetic

Spaceflight is detrimental to several genetic processes.

Meiosis (reproduction). Fertilization and gastrulation are negatively impacted.[64,75] Spermatogenic cells and ovarian follicles are sensitive to HZE particles (increasing the possibility of premature ovarian failure). Microgravity decreases testosterone synthesis and spermatogenesis. Fertilized ovum implantation is jeopardized by increases in sperm swim rate and apoptosis,[35,86] as well as vaginal acidity. Reductions in vaginal wall lubrication, endometrial thickening, and FSH-mediated ovulation occur.

Gestation and fetal development are compromised as well. Tadpoles on the SL-J mission initially grew normally but were unable to inflate their lungs.[99] Pregnant rats flown into space gave birth, but the pups couldn't attach themselves to their mother's nipples in microgravity and were cannibalized. Alterations in fetal cardiomyogenesis, calcium bone mineralization, and the development of the choroid plexus, vestibular, and sensorimotor systems might occur in microgravity. Human maternal risks include polyhydramnios and diminished progesterone production. Microgravity allows the fetus to sit higher in the womb, pressing upon the mother's diaphragm and making respiration more difficult. It may interfere with dropping by week 39.

Mitosis (carcinogenesis). NASA radiation exposure limits are based on Risk of Exposure Induced Death (REID) values: cumulative doses that will keep an astronaut's risk of developing fatal cancer to ≤3%—but there are uncertainties related to space radiation cancer risk predictions.[18] GCR exposure estimates derived from dosimeters aboard the Mars Science Laboratory predicted that human crewmembers could exceed career radiation exposure limits during just the in-flight portions of a Mars journey. The addition of SPE transit and Mars surface doses would put cancer risk into a more dangerous range.

Apoptosis (aging). During a long-duration ISS mission, the NASA Twins Study[25] demonstrated telomere shortening akin to changes observed with aging. Humans in space develop aging features accelerated tenfold from normal senescence on Earth.

Spaceflight and aging both result in decreased thermoregulation, plasma volume, thirst, visual and hearing acuity, taste sensation, immune competence, antibiotic sensitivity, aerobic capacity, cardiac output, baroreflex sensitivity, arterial elasticity, endothelial thickness and nitrogen oxide generation, epidermis, joint collagen, height, bone density, skeletal muscle mass, protein synthesis, strength and explosive power, growth hormone and GH exercise-response, testosterone, vitamin D3, insulin sensitivity, gastric motility and gut transit time and absorption, urinary continence, wound healing, cerebral mass and blood flow and oxygenation, sleep and circadian cyclicity, posture, balance, coordination, movement, and reaction time. In both cohorts, body fat replaces muscle and infiltrates the liver, and there are increases in renal stones, orthostatic hypotension, latent viral infection reactivation, aching joints, back pain, tender soles, and vertebral compression and bone fractures.

Immune System

The physical environment of space exacts a toll on innate and acquired vertebrate immune systems.[14] Innate immune cells are more radioresistant than acquired phenotypes. But T and B lymphocytes, the main components of acquired immunologic memory, are exquisitely radiosensitive. They eliminate other cells (damaged from viruses or carcinogenesis) and invading microbes with more precise responsiveness than innate system reactions.

Microgravity causes additional acquired immune dysregulation that consists of altered innate and acquired interactions, cytoskeletal disruption (with a reduction in peripheral monocyte-endothelial cell adhesion and tissue migration secondary to decreased CD26L and HLA-DR surface marker expression), and peripheral leukocyte number and distribution change[13,15] (a drop in specific subpopulation function; elevated granulocyte numbers but decreased function; less neutrophil ROS production during oxidative burst and phagocytosis; and fewer eosinophils). Impaired differentiation and maturation of all immune cells result in premature immunosenescence and overactive immunity syndromes (e.g., increased allergy, asthma, eczema, autoimmunity, and cancer risk).

B cells are affected by microgravity with reduced lymphopoiesis, proliferation, subset distribution, generation, frequency, antigen-specific response, immunological memory, and delayed hypersensitivity responses to recall antigens. Thymic and splenic atrophy and dysfunction result in reduced T cell development, output, and function (with changes in TCR signaling from cytoskeletal disruption; direct CD3/CD28-driven T-cell activation and response; monocyte accessory cell and macrophage[85] malfunction; cytotoxic NK cell and blastogenic activity; concanavalin A-induced mitogen proliferation and lymphocyte response; and mislocalization of Krt5 TECs in

the thymic cortex). Circulating TLR2, TLR4, TLR6, and LPS-induced cytokine production and reactivity is reduced. A signaling shift toward the Th2 cell population leads to viral shedding and decreases in IL-1, IL-2, and IL-2 receptors; it also leads to increases in TNFα, IL-4, IL-6,[82] IL-8, IL-1 receptor antibody, thrombopoietin, vascular endothelial growth factor, IFN-γ, and leukocyte recruitment mediators.

Microbes respond differently to space stressors than humans.[63,84] Spaceflight augments microbial pathogenicity through changes in spacecraft commensal populations (a rise in fungal colonization causes an amplified astronaut immune response to fungal antigens); increased infectivity and microbial anatomic breach from impaired innate mechanisms (e.g., reduced monocyte *E. coli* phagocytosis); augmented virulence of *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Aspergillus*; and biofilm formation from bacteria (*Acinetobacter*, *Sphingomonas*, *Corynebacterium*, *Burkholderia*, *Bacillus*, and *Klebsiella*) and fungi (*Penicillium*, *Aspergillus*, *Cryptococcus*, and *Rhodotorula*).[19] Biofilms are responsible for 80% of chronic and recurrent infections and are associated with prostatitis, rhinosinusitis, otitis media, urinary tract infection, endocarditis, periodontitis, and infectious kidney stones. They also impair heat transfer and cause ISS equipment corrosion and mechanical blockages.

In the setting of tight crew proximity, more antibiotic resistance increases the risk of secondary infection. Decreased CD8+ cytotoxic T cell activation facilitates reactivation of latent viral infections such as human herpes, Epstein-Barr, varicella zoster, and cytomegalovirus.

Blood

Hematological. Upon entering space, all astronaut red blood cells are terrestrial born; after 120 d in flight, all RBCs are space born. Both erythrocyte populations are destroyed.[90] Hemolysis increases 54% over baseline as a function of space exposure duration,[91] independent of fluid shifts, EPO levels, and the RBC production environment. The mechanism(s) of this extravascular anemia are unknown. Consequences include elevated CO levels; decreased peripheral O_2 delivery; iron-mediated oxidative damage; free Hgb and heme-mediated endothelial dysfunction; increased compensatory RBC production; Hgb concentration, viscosity, and rheological burden; and higher nutritional demands. Effects can persist a year after long-duration space exposure and the anemia does not respond to exercise or nutritional countermeasures.

Coagulation. In 2019, an incidental obstructive left internal jugular venous thrombosis was identified in an astronaut 2 mo into his ISS mission. A nonobstructive jugular clot was identified in another crewmember.[54]

Virchow's triad, the three factors that contribute to venous thromboembolism, is as relevant in microgravity as on Earth. Blood stasis occurs in microgravity. Headward fluid shift is confounded by a lack of upper body venous valves impeding cephalad blood drainage. Jugular cross-sectional area increases by a factor of 7, JVP by a factor of 4.[59] Stagnant and retrograde jugular venous flow occurs in more than 55% of ISS crewmembers with a decrease in musculovenous pump activity. Hypercoagulability increases with a rise in fibrinogen synthesis rate, fibrinogen α-chains, D-dimer levels, thrombin-antithrombin complexes, prothrombin F1+ F2, and modulator activation.[45] Vessel wall remodeling from rheological changes, reduced venous flow, and proinflammatory change and oxidative stress lead to increased local tissue injury, thrombotic markers, procoagulant molecule expression, extracellular matrix, low-density lipoprotein (LDL) uptake, and lipid synthesis with enhanced atherogenesis.[45]

Integument

Skin. Three categories of skin changes occur in spaceflight. Epidermal stratum corneum thins secondary to an increase in cell molting time and diminished barrier function, hydration, and elasticity. Sloughing and coarsening make for "rough hands and soft feet." Dermal collagen content increases and changes in ECM gene expression result in matrix degradation. Melanin content is reduced. Skin microbiota/commensals are recolonized with uncommon microorganisms (e.g., ascomycetous *Cyberlindnera jadinii*).

A significant proportion of ISS crew (40%) develop skin rashes (1.12 rashes per flight year vs. 0.044 per year on Earth) across a spectrum of conditions.[22] Diminished ambient humidity and temperature lead to dry skin (xerosis) and acute flares of atopic dermatitis/eczema (requiring sedating antihistamine and systemic corticosteroid medication). Contact dermatitis occurs secondary to irritants (micropore tape, fiberglass, beta cloth, ECG chest wall electrode patches, gloves, face masks, and headphones).

Additional factors predispose certain individuals to skin infections. Air filtration is constant. Hygienic use of wet wipes, no-rinse shampoos, and soaps, as well as increased contamination from skin shedding, result in a rash incidence 5× higher than found in submariners. Immune system dysregulation results in delayed wound healing. Habitat microbiota are characterized by higher virulence, antibiotic resistance, and faster growth (due to low shear stress and low turbulence). *S. aureus* and pathogenic fungi colonization increase (with crew transmission). Mir spacecraft were colonized with *E. coli*, *Serratia marcescens*, *Legionella*, spirochetes, and dust mites. Reductions in *Gammaproteobacteria* populations are associated with inflammation and allergy sensitization.

Four main types of skin infection present more commonly. Latent viral reactivation has already been described above. Cellulitis occurs more readily from *Staphylococcus* and *Streptococcus* colonization. Treatment of acne vulgaris is more difficult because of the need to avoid minocycline and isotretinoin side-effects. Antifungal therapy of dermatophytosis is also limited; gels and powders are restricted because of inhalation/flammability risk.

Other dermatologic conditions are notable. Skin doses from SPEs are 510× higher than seen by internal organs. The severe

erythema, blistering, and necrosis of cutaneous radiation syndrome (CRS) can occur from a single exposure of ionizing radiation >3 G_y. U.S. astronauts have 3× the risk for localized basal and squamous cell carcinoma. Urticarial allergic reactions may arise due to decompression sickness. The treatment for psoriatic exacerbations is limited.

Synovial joints/cartilage. Most of what we know about the effects of spaceflight on cartilage[24,79] come from 30-d murine studies on BION-M1.[46,47] Articular cartilage (AC) and sternal fibrocartilage (SC) respond differently to microgravity:[23] SC is loaded by cyclical lung expansion and, since mice continue to breathe in microgravity continuously, no cartilage breakdown occurs; cyclical compressive loading of AC in microgravity causes damage at the point of greatest cartilage-to-cartilage contact during weight-bearing. Radiation has a compounding effect on cartilage damage;[102] cartilage has limited capacity for repair and microgravity induces a flexor bias in joint position.

Bone. It is energetically costly to maintain a dense skeleton for fewer weight-bearing activities. Healthy astronauts lose bone mass 10× faster than post-menopausal women on Earth. Vertebral and lower limb skeletal sites are especially susceptible to bone loss from microgravity mechanical unloading. Diminished osteoblast production, cytoskeletal tensegrity, adhesion, increased osteoclast activity, sclerostin expression, and loss of 'piezoelectric strain' occur.[42] Elevated serum calcium decreases circulating PTH, renal active vitamin D activation, and calcium gut absorption, causing a rise in urine calcium and nephrolithiasis propensity.

Expanded iron stores create unbalanced bone remodeling mediated by Fenton reaction oxidative stress. Net endogenous acid production foods, high sodium, and an elevated animal protein to potassium ratio increase endogenous acid production (which is neutralized by the $CaCO_3$ released during bone resorption). Spacecraft CO_2 concentrations are 10× higher than on Earth.

Rodents exposed to space-relevant doses of radiation experience accelerated resorption (especially cancellous bone). The cumulative result is an increased fracture risk which persists postflight.[37,96,97] Trabecular architecture may never return to normal. Because muscle mass and strength recover faster than bone, the risk of injury to tendon insertion sites and avulsion fractures is increased.

Cardiovascular

Hemodynamic/structural adaptations. In short-duration spaceflight, hydrostatic gradient loss results in a 2-L cephalad fluid shift, higher upper body intravascular pressures, central vasculature distension, and increased venous return. Astronauts experience neck vein congestion, "puffy" faces, "stuffed" noses, and "chicken legs."

Elevated cardiac preload causes a 20% distension of cardiac chamber size with increased left ventricular end-diastolic volume, carotid, aortic, and cardiac baroreceptor stimulation; ANP-induced vasodilatation with a decrease in systemic vascular resistance (SVR); and renin-angiotensin-aldosterone system inhibition.

Hypovolemia results from a 10–15% drop in plasma volume (PV) with intravascular and extracellular fluid moving transcapillary to interstitial and intracellular compartments, exacerbated by smaller fluid intake secondary to motion sickness and diuresis.

A reflex 46% increase in stroke volume (SV) and 24% rise in cardiac output (CO) occurs without any change in mean arterial pressure or heart rate. The drop in apparent central venous pressure (CVP) is due to the hydrostatic pressure column loss effect being less than the lung/thoracic cage expansion effect:

$$\downarrow CVP = \uparrow TCVP + \downarrow\downarrow IPP$$

where TCVP = transmural CVP and IPP = intrapleural pressure from thoracic expansion.

The heart atrophies by 10% after a 10-d spaceflight (secondary to decreased metabolic demand and O_2 uptake), changing its configuration from elliptical to spherical.

In long-duration spaceflight (≥6 mo), the effective circulating PV is still reduced 10–15%, and systolic, diastolic, and mean blood pressures have dropped by 8 mmHg, 9 mmHg, and 10 mmHg, respectively, which increases pulse pressure, elevates SV by 35% and CO by 41%, and drops SVR by 39%.

Heart rate is lower or unchanged. No upregulation of autonomic sympathetic activity occurs. LV mass decreases by 12% ± 6.9% with a concomitant drop in preload, contractility, and afterload.[34,38,94]

Cosmic radiation and the heart. Terrestrial murine cardiac studies of orbital plane entrance GCR radiation equivalents have demonstrated coronary artery fibrosis, smooth muscle degeneration, and extracellular deposition 15 mo after single dose 0.1–0.2 G_y exposure, elevated aortic stiffness and ex vivo aortic tension 8 mo after single dose 1 G_y exposure, and increased aortic lesions, carotid intima-media thickening, and atherosclerosis after single dose 2 G_y exposure.

Cardiovascular disease deaths are greater than fourfold in low Earth orbit astronauts, and more than fivefold in Apollo lunar astronauts.[8] For a 40-yr-old man on a 1000-d exploratory Mars mission, the estimated cumulative radiation exposure of 0.5–1.0 Sieverts will result in a 1.3–13% higher lifetime risk of cardiovascular death.[4,34]

Clinical Consequences

Reduced exercise and work capacity. Total peripheral oxygen delivery is the product of cardiac output × arterial oxygen content (the amount of oxygen bound to hemoglobin plus the amount of oxygen dissolved in arterial blood):

$$\dot{V}O_2 = \left[HR \times SV\right] \times \left[1.34 \times Hgb \times SaO_2 + 0.003 \times P_aO_2\right]$$

In spaceflight:

$$\downarrow \dot{V}O_2 = \left[\downarrow HR \times \downarrow SV\right] \times \left[1.34 \times \downarrow Hgb \times SaO_2 + 0.003 \times P_aO_2\right]$$

The sum effect of these reductions results in a decline in peak aerobic exercise capacity (with decreased convective and diffusive oxygen transport), a drop in anaerobic threshold, and impaired thermoregulation. Deconditioning is greater in those who start with higher maximal aerobic capacity.[29]

Spaceflight-associated neuro-ocular syndrome (SANS). Found in 66.7% of astronauts who have undergone long-duration space flight missions in microgravity,[50,101,108] the threat of spaceflight blindness is serious enough for NASA to label SANS a top "Red Risk" danger that crews will face during deep space missions.[74]

The mechanism of SANS visual impairment is multifactorial and muddled. Symptoms of headache, pulsatile tinnitus, diminished visual acuity, and scotomata occur, along with: ophthalmoscopic signs of cotton wool spots, nerve fiber glutting, choroidal folds, and optic disc edema (21.2% with class 3–4 papilledema); increased cerebral free water volume; and increased biochemical markers of brain damage and morphological brain changes.

Although some ocular structural changes from SANS may persist for years after spaceflight, no crewmember has yet experienced permanent blindness postflight.

Neck vein thrombosis. The ISS internal jugular vein thrombus was treated successfully. If future astronauts require prophylactic anticoagulation, what additional risks to space habitability will exist in bleeding-prone crew with a high fracture hazard potential?

Postflight orthostatic intolerance/reacclimation. Post-flight syncope from decreased PV, SVR, vasoconstrictor responsiveness, and baroreceptor function despite aggressive pre-re-entry fluid-loading protocols[65] remains problematic. In 20–30% of short-duration flights, astronauts are unable to maintain upright body position; this figure rises to 83% for astronauts returning from long-duration missions. Landing on another celestial body could result in catastrophic consequences.

Arterial remodeling When blood pressure is low, endothelial cells secrete vasoactive molecules (angiotensin II, endothelin-1, and ROS) that increase vasoconstriction.

In microgravity, hydrostatic pressure gradient loss results in transmural pressure redistribution along vessel walls, cellular remodeling, and vascular functional changes.[62,70]

Arteries in the lower leg undergo a decrease in intima-media thickness (IMT), cross-sectional area, and adrenergic responsiveness, as well as an increase in endothelial vascular smooth muscle cell (VSMC) nitrogen oxide release and vasorelaxation.

Upper body arteries undergo more pronounced changes. Increased carotid and femoral IMT (20% after 1 yr on ISS) and carotid cross-sectional area (10%) result from augmented mesenchymal stem cell differentiation to tunica media VSMCs.

Extracellular Ca_2+ influx occurs through upregulated voltage-dependent Ca_2+ channels. Activated calcineurin translocated to the nucleus results in VSMC dedifferentiation, proliferation, remodeling, and loss of "contractile" phenotype.

Increased ECM production, cell apoptosis, NO release, cellular cytoskeleton damage (from microgravity mechanical unloading), and vascular stiffness on the order of 17–30% also occur. The endothelium undergoes more oxidative stress, as well as inflammation multicellular spheroid formation and apoptosis.

Monocyte chemoattractant protein, chemokine ligand 5 protein CCL5, and neutrophil gelatinase-associated lipocalin promote neo-angiogenesis.

Spaceflight-associated CVD risk factors of radiation exposure, elevated total cholesterol, oxidized LDL, insulin, iron, inflammation, circulating catecholamines and psychosocial stressors, and changes in diet, exercise, and sleep routines promote accelerated atherosclerosis.

Cardiomyopathy. Microgravity-induced deconditioning and remodeling from strain/stress and pressure–volume changes results in cardiac sphericity; decreased LV compliance and diastolic suction lead to diastolic dysfunction and reduced early ventricular filling that lowers SV. The drop in cardiac workload causes an LV mass reduction of 10–20% (~12% after just 10 d onboard). Cardiomyocytes are also sensitive to ionizing radiation.[60]

Disturbances of automaticity, rhythm, and conduction. Electrocardiac disorders that have been observed in spaceflight include bigeminal rhythm on the Moon's surface (Apollo 15); 14 beats of nonsustained ventricular tachycardia (MIR); persistent tachyarrhythmia (MIR-2 EVA); ventricular couplets and triplets; ST-segment and T-wave changes during physical exertion; HR variability loss from augmented vagal output (associated on Earth with an increased Sudden Cardiac Death-Hazard Ratio of 2.12); QTc-prolongation (associated on Earth with polymorphic ventricular tachycardia); and transient AV-block, supraventricular premature beats, ventricular premature beats, and junctional rhythm (especially during lower body negative pressure sessions). Increased left atrial size and changes in p-wave morphology that may predispose to atrial fibrillation have been observed postflight.[44]

Respiratory

No structural adaptive changes are observed in spaceflight and no degradation in lung function is seen upon return to Earth. However, there are potential sources of lung damage in flight.[77] These consist of strong SPE radiation causing acute inflammation, which predisposes to pulmonary fibrosis, decompression stress, and pathogenic microbe inhalation (murine studies demonstrated compromised *Klebsiella pneumoniae* clearing, with increased morbidity), and particulate toxic dust/aerosol exposure, which, by settling out along peripheral airspaces, increases toxicity.

GI

Gut. The first symptoms of acute radiation syndrome (ARS) manifest in the gut. Radiation induces GI serotonin secretion which binds to brain receptors that mediate vomiting. This can be problematic, especially in EVA suit confinement.

With that said, spaceflight affects the entire GI tract.[107] Mastication and deglutition are compromised because the jaw opens with gravity on Earth and keeping the mouth shut requires energy in microgravity. There is a decrease in mandibular bone density and salivary amylase production, as well as masseter muscle atrophy and an increase of both metalloproteinases and secretory immunoglobulin A. Smad signaling pathway activation causes proliferation and differentiation of human periodontal and dental pulp stem cells.

The stomach exhibits slow wave motor dysfunction, hypersecretion, and impaired mucosal barrier function. The intestinal tract sees changes in digestion, hemodynamics and lymphodynamics, intestinal mucosal permeability, and intestinal flora and microecology.

The GI microbiome is a virtual organ with over 1000 species of bacteria. It facilitates carbohydrate fermentation and absorption, metabolic activity (salvaging energy from indigestible compounds), vitamin synthesis, gut and systemic immune regulation, epithelial barrier integrity, competitive repression of pathogenic microbes, and angiogenesis.

Spaceflight alters crew space gut microecology[81,92,98] with a decrease in protective *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium prausnitzii*, and *Akkermansia;* an increase in pathogenic *Serratia marcescens*, *Staphylococcus aureus*, *S. hominis*, *S. haemolyticus*, *S. epidermidis*, *Bacteroides*, pathogenic *E. coli*, *Clostridium difficile*, *Salmonella typhimurium*, *Alloprevotella*, *Parasutterella*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus fumigatus*; transformation of symbionts to pathobionts (increasing intestinal permeability and opportunistic virulence and pathogenicity); and an increase in antibiotic resistance.

The liver is a radiation-sensitive organ that also responds to microgravity[41,61] with glycogen accumulation, altered plasma protein production (upregulated gluconeogenetic polypeptide and downregulated lipid peroxidation stress response protein synthesis), and elevated bile acid secretion which decreases retinol (vitamin A) secretion. CYP-450 monooxygenase activity is halved, impairing drug metabolism. There is an increase of oxidative stress and a decrease in S_{16}-containing antioxidants. Decreased portal vein blood flow and first-pass metabolism predisposes to portal endotoxemia/hepatocyte apoptosis. Diminished hepatic lipid metabolism leads to a 19% rise in serum cholesterol and could contribute to NAFLD/NASH metabolic liver disease/cirrhosis.

Murine research has shown that the pancreas becomes atrophic in spaceflight, causing elevated plasma glucose, insulin and C-peptide secretion, glucagon, and heat shock protein HSP70 expression.

GU

Microgravity causes interstitial edema, alterations in urinary protein composition, and decreased renal blood flow and urinary albumin and sodium excretion.[76] Examination of renal histopathology in rats after exposure to simulated microgravity revealed glomerular atrophy, interstitial edema, and degeneration of renal tubular cells.[52]

Decreased fluid intake, hypercalciuria, and nanobacteria could facilitate CaC_2O_4 renal stone formation. Urodynamic changes and anticholinergic therapy for space sickness predispose to acute urinary retention. Urinary tract infections are more frequent.[5]

Neuromuscular

Ocular. In addition to SANS, astronauts are prone to cataracts, foreign bodies, and corneal abrasions.

Olfactory/gustatory. Spaceflight smells like a mélange of welding fumes, burnt steak (or burnt almond cookies), walnuts, motorbike brake pads, a pile of wet clothes after a day in the snow, gunpowder, brimstone, and sherry secondary to polycyclic aromatic hydrocarbons and ozone.

Alterations of taste are related to dry air, background noise masking, stress, circadian dysfunction, rehydrated food, and nasal and sinus stuffiness from cephalic fluid shifts. Food tastes as bland as "eating with a head cold."

Polyreceptive control of sensorimotor function. Despite vestibular nuclei plasticity, simultaneous afferent signal conflict from gaze center, oculomotor, corticocerebellar, and proprioceptive input can cause symptoms of disturbed equilibrium, balance, locomotion, and fine motor control in microgravity.[10,73,88]

Astronauts have a 75% incidence (92% in long-duration missions) of space adaptation syndrome. Symptoms include apathy, depression, and disinclination for work. They can persist or reoccur up to 14 d during or after spaceflight. Sensory conflict and limbic neural mismatch may both contribute to causation.[49]

CNS. The effects of ionizing radiation on the mammalian central nervous system have been well-studied.[43,72,80] On a structural level, neurogenetic inhibition of stemlike neural precursor cells (NPCs), astrocytes, and oligodendrocytes is observed along with a decrease of dendrite complexity and dendritic spine numbers. A reduction in capillary numbers and barrier function, microvessel segments, and endothelial cells leads to blood-brain barrier compromise. MRI features of widespread tissue changes without necrosis are noted.

Molecular and cellular alterations result from oxidative stress accompanied by enzymatic changes, proinflammatory cytokine production, and proliferation of microglial and astrocyte activation markers. Radiation causes peripheral monocyte and T lymphocyte infiltration, reduced microvascular adhesion molecules, and changes in synaptic protein and glutamate-gated ion channel levels, acetylcholine and dopamine pathways, and genetic expression.

Radiation impacts electrophysiological function. Decreased resting membrane potential, input resistance, and long-term potentiation lead to impaired cell excitability, reduced memory formation capacity, and synaptic plasticity.

Behavioral consequences of radiation exposure include attention/vigilance, reaction time, learning, memory, cognition, mood and emotional control, and social interaction. There

are uncertainties in space radiation CNS risk prediction based on animal models.

Sleep/circadian cyclicity. Human circadian timing system evolved in 1 g. Spaceflight is associated with a diminution in sleep time, slow-wave sleep, and REM sleep; REM latency; and an increase in the latent period for falling asleep and number of arousals. Hypnotic use is pervasive among astronauts despite any performance impairment of potentially hazardous activities requiring complete mental alertness or motor coordination that may occur the day following ingestion.

Cognition. The "Space Fog" of short-duration spaceflight sits on a continuum of cognitive dysfunction which could potentially culminate in the "Space Brain" and dementia of long exposure.[12]

Behavioral. The explorer Richard Byrd took only two coffins, but 12 straightjackets on his expeditions to Antarctica in the 1930s. NASA ranks behavioral risk second only to radiation exposure as a threat to successful exploration class missions. Dysfunctional behavior can result from neurobehavioral, cognitive, psychological, or psychosocial causes from mission-related, individual, cultural, family, and interpersonal and crewmember interaction factors.

Muscle. In microgravity, site-specific antigravity muscle atrophy progresses quickly. Plantar flexor peak force lessens by 20–48% after 6 mo of spaceflight and the number of Soleus type I fibers decreases by 21% after only 17 d of spaceflight (STS-78 mission Shuttle Astronaut B had myofibril atrophy and mitochondrial rounding in postflight soleus biopsies corresponding to decreased force and increased shortening velocity of single Ca^{2+}-activated muscle cells).[100]

Muscle strength is lost because of unloading (disuse atrophy), diminished neural drive (denervation atrophy), and increased protein catabolism from stress and undernutrition.[1,89] Astronauts become taller in microgravity and the lengthening spine becomes a source of mechanical back pain and disk herniation. Weakened tendons and ligaments predispose to ankle injury and Achilles tendon rupture.

Other Illnesses

Microgravity causes more dental problems (barodontalgic tooth pain, caries, periodontal disease, gingivitis, and periapical abscesses in Shuttle-MIR cosmonauts), ENT disorders (barotitis, sinus headache, epistaxis, and deafness), and traumatic injury. Terrestrial human illness could also occur de novo in space. Combined effects can be additive, subtractive, or synergistic. Return to Earth from long-duration missions may be difficult or impossible.

COUNTERMEASURES TO THE HAZARDS OF LTS/LIS

The external vacuum environment and temperature extremes of space can be easily mitigated with a heated gas ratio atmosphere pressurized to nominal sea level on Earth.

Passive bulk shielding is the only current practical means of limiting space radiation exposure,[58] but the mass requirements and cost are high and secondary nuclear interactions can be deleterious.[66] Other radiation countermeasures, radioprotectors, and radiomodulators[93] have dubious benefit.[68] Active treatment of radiation exposure is reserved for situations which are usually already unfortunate.

Countermeasure advances in the reduction of negative effects from microgravity include the mobile lower body negative pressure (LBNP) "gravity" suit,[3,31] aerobic and resistance (ARED) exercise,[83] intermittent short-arm centrifuge use,[48] and their combinations.

The LBNP suit is limited by individual specificity, changing astronaut biometrics during spaceflight, and intermittent utilization cycles of reconditioning and deconditioning. The omnidirectional lower body forces are not the same as the Z vector forces in 1 g.

Aerobic exercise is insufficient to maintain upright exercise capacity, orthostatic tolerance, or musculoskeletal mass and function, and it diverts critical crew time from operational tasks.

Exercise countermeasures do not produce the same level of mechanical loading possible on Earth—ISS workouts are limited to skeletal muscles that move the limbs and torso, but most muscle groups cannot be exercised (small face and finger muscles get weaker in spaceflight). Resistance exercise also heightens SANS risk.

Intermittent short arm centrifuge use ± exercise causes unpleasant vestibular, Coriolis and cross-coupling effects, motion sickness, lateral strains on exercising lower body joints, and low footward forces. None of these options will prevent most of the adverse consequences of microgravity. A more consistent continuous solution requires an ideal level of sustained artificial gravity. Until evidence accumulates to the contrary, the most reasonable level is the most conservative (i.e., 1 G). Nothing has changed to soften or dislodge this recommendation since the 1975 NASA Space Settlements Study.

The most practical achievable way of generating artificial gravity with current technology is with centrifugal force.[57] The next most pressing issue to resolve is rotation rate. We have a plethora of experimental data on terrestrial rotation rate tolerability with the subject longitudinal axis prone in 1 G, and parallel to the axis of rotation during locomotion. Some investigators have suggested that these results infer adaptability to faster rotation rates. But in space, the longitudinal body axis will be orthogonal to the axis of rotation, and Coriolis forces will cause motion sickness and increased injury risk at lower angular velocities. The answer to the rotation rate question will have to wait for human studies in space. Until then, the optimal tolerable rotation rate to produce 1 G is 1 rpm.[36,104]

Despite the development of sophisticated preflight, inflight, and postflight countermeasures to the potential negative effects of isolation and confinement, mental health becomes more brittle as a function of mission duration. There are also issues of physician–patient confidentiality, and preflight decisions regarding single gender crews (to minimize complications of crew cohesion/performance), compulsory appendectomies

(to eliminate risk of acute appendicitis), and mandatory blood group compatibility (to provide a blood source for limited transfusion).

Some specific organ system countermeasure progress has occurred to reduce bone and muscle loss, nephrolithiasis, postflight orthostatic hypotension, and circadian dyscyclicity. The study of human physiology in space is an ongoing work in progress, but it is unclear if this research will find robust solutions to compensate for our fragility and enable us to undertake long-term missions or live in space within an envelope of acceptable risk.

WHAT WE'VE LEARNED SINCE THE 1975 NASA SPACE SETTLEMENTS STUDY

The Stanford Torus Study was published almost five decades ago, well before several new areas of pathophysiological concern had emerged. The Human Genome Project and advances in our understanding of gene expression have been revolutionary. We have a more profound appreciation of cell death pathways secondary to ionizing irradiation; cellular and organelle microstructural, functional, maturation, differentiation, and proliferation changes secondary to microgravity; oxidative stress; adaptive immunity dysregulation, microbial pathogenicity, viral reactivation, and biofilms; spaceflight-associated hemolytic anemia and venous thrombosis; endothelial dysfunction, atherosclerotic coronary artery disease, and LDL dyslipidemia; SANS and the oculo-cerebral glymphatic system; and 'Iome' connectivity (microbiomes, genomes, epigenomes, proteomes, transcriptomes, metabolomes, immunomes, and the space exposome). However, the life support standards and recommendations for long-term spaceflight and living in space remain the same.

CONCLUSION

Dr. Louis Friedman, cofounder of The Planetary Society, maintains that space travel by humans will stop at Mars.[26,27] I asked him whether this conclusion derived from the physical or the physiological limitations of long-term spaceflight.

> "Neither actually. It's because of the pace of evolution—human and technological. Human space travel and human space capability is evolving VERY slowly. Even if humans get to Mars in this half century, we certainly won't explore it much within the whole century. In that same time period, technology will continue to advance rapidly with stuff we can't predict in robotics, AI, VR, and other information processing.
>
> So it's kind of a dual conclusion—Mars will keep us busy for a long time… and by then there will be no reason and no advantage and no social interest in humans going beyond when they will already be doing virtually."[28]

Dragging a few individuals of our fragile species interstellar inside complex, massive rotating, radiation shielded starships may prove more cumbersome and expensive (and vainglorious) than the virtual exploration and colonization we could achieve with rapidly evolving parallel technologies.

It may be possible to re-engineer the human genome to resist microgravity-related bone loss with a G171V mutation[110] and muscle atrophy with myostatin gene K153R alteration,[89] and even protect against cosmic radiation effects with CRISPR/Cas9-mediated genome editing.[53] We might consider sending "seedships" to Goldilocks or robotically terraformed planets, using generational succession, synthetic hibernation,[78] or robot-frozen zygote missions. But why would we want to spread ourselves through the universe like a time capsule when we could use our SMARTS (Solar sails, Miniaturization, AI, Robots, Telescopes, Solar gravitational lenses)?

Being unable to travel and live in deep space with our current protoplasm will not destroy our dreams and enthusiasm to inhabit the cosmos that surrounds us, but it may encourage us to take better care of the one place in the universe we know can sustain life as we know it.

There may also be extraterrestrial intelligent AI robots heading our way, despite the silence of the Fermi Paradox.[23] If (or when) someone from somewhere comes calling, we may be working from home.

ACKNOWLEDGMENT

Dedicated to the late MIT Professor Laurence 'Larry' R. Young.

Financial Disclosure Statement: The author has no competing interests to declare.

Author and Affiliation: Lawrence H. Winkler, M.D., FRCPC (Internal Medicine), FACP (Internal Medicine), Assistant Professor of Medicine, University of British Columbia (Retired).

REFERENCES

1. Adams GR, Caiozzo VJ, Baldwin K. Skeletal muscle unweighting: spaceflight and ground-based models. J Appl Physiol (1985). 2003; 95(6): 2185–2201.
2. Afshinnekoo E, Scott RT, MacKay M, Pariset E, Cekanaviciute E, et al. Fundamental biological features of spaceflight: advancing the field to enable deep-space exploration. Cell. 2020; 183(5):1162–1184. Erratum in: Cell. 2021 Nov; 184(24):6002.
3. Ashari N, Hargens AR. The mobile lower body negative pressure gravity suit for long-duration spaceflight. Front Physiol. 2020; 11:977.
4. Baran R, Marchal S, Campos SG, Rehnberg E, Tabury K et al. The cardiovascular system in space: focus on in vivo and in vitro studies. Biomedicines. 2021; 10(1):59.
5. Baran C, Erkoç M, Ötünçtemur A. The place of urology in aerospace medicine; a new horizon. Eur Arch Med Res. 2022; 38(1):1–4.
6. Blue RS, Bayuse TM, Daniels VR, Wotring VE, Suresh R, et al. Supplying a pharmacy for NASA exploration spaceflight: challenges and current understanding. NPJ Microgravity. 2019; 5(1):14.
7. Boddy J. From shrinking spines to space fungus: the top five dangers of space travel. Science. 2006. [Accessed March 30, 2023]. Available from https://www.science.org/content/article/shrinking-spines-space-fungus-top-five-dangers-space-travel.

8. Boerma M, Nelson GA, Sridharan V, Mao XW, Koturbash I, Hauer-Jensen M. Space radiation and cardiovascular disease risk. World J Cardiol. 2015; 7(12):882–888.
9. Bradbury P, Wu H, Choi JU, Rowan AE, Zhang H, et al. Modeling the impact of microgravity at the cellular level: implications for human disease. Front Cell Dev Biol. 2020; 8:96.
10. Carriot J, Mackrous I, Cullen KE. Challenges to the vestibular system in space: how the brain responds and adapts to microgravity. Front Neural Circuits. 2021; 15:760313.
11. Carter K, Campbell J, Shoemaker P, Dichiara E, Patel N, Caruso J. Dietary needs, approaches and recommendations to meet the demands of future manned space flights. recent progress in nutrition 2022; 2(1):005.
12. Cherry JD, Liu B, Frost JL, Lemere CA, Williams JP, et al. Galactic cosmic radiation leads to cognitive impairment and increased aβ plaque accumulation in a mouse model of Alzheimer's disease. PLoS One. 2012; 7(12): e53275.
13. Cogoli A. The effect of hypogravity and hypergravity on cells of the immune system. J Leukoc Biol. 1993; 54(3):259–268.
14. Crucian BE, Choukèr A, Simpson RJ, Mehta S, Marshal G, et al. Immune system dysregulation during spaceflight: potential countermeasures for deep space exploration missions. Front Immunol. 2018; 9:1437.
15. Cromer WE, Zawieja DC. Acute exposure to space flight results in evidence of reduced lymph transport, tissue fluid Shifts, and immune alterations in the rat gastrointestinal system. Life Sci Space Res (Amst). 2018; 17:74–82.
16. Cucinotta FA, Durante M. Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings. Lancet Oncol. 2006; 7(5):431–435.
17. Cucinotta FA, Kim MH, Chappell LJ, Huff JL. How safe is safe enough? Radiation risk for a human mission to Mars. PLoS One. 2013; 8(10): e74988.
18. Cucinotta FA. A new approach to reduce uncertainties in space radiation cancer risk predictions. PLoS One. 2015; 10(3):e0120717.
19. Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer A, et al. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One. 2007; 2(5):e457.
20. da Silveira WA, Fazelinia H, Rosenthal SB, Laiakis EC, Kim MS, et al. Comprehensive multi-omics analysis reveals mitochondrial stress as a central biological hub for spaceflight impact. Cell. 2020; 183(5): 1185–1201.e20.
21. Dello Russo C, Bandiera T, Monici M, Surdo L, Yip VLM, et al. Physiological adaptations affecting drug pharmacokinetics in space: what do we really know? A critical review of the literature. Br J Pharmacol. 2022; 179(11):2538–2557.
22. Dunn C, Boyd M, Orengo I. Dermatologic manifestations in spaceflight: a review. Dermatol Online J. 2018; 24(11).
23. Fitzgerald J, Endicott J, Hansen U, Janowitz C. Articular cartilage and sternal fibrocartilage respond differently to extended microgravity. NPJ Microgravity. 2019; 5(1):3.
24. Fitzgerald J. Cartilage breakdown in microgravity—a problem for long-term spaceflight? NPJ Regen Med. 2017; 2(1):10.
25. Garrett-Bakelman FE, Darshi M, Green SJ, Gur RC, Lin L, et al. The NASA twins study: a multidimensional analysis of a year-long human spaceflight. Science. 2019; 364(6436):eaau8650.
26. Friedman LD. Human spaceflight: from Mars to the stars. Tucson (AZ): University of Arizona Press; 2015.
27. Friedman LD. Oh the places we won't go: humans will settle Mars, and nowhere else. Sci Am. 2015. [Accessed March 28, 2023]. Available from https://www.scientificamerican.com/article/oh-the-places-we-won-t-go-humans-will-settle-mars-and-nowhere-else-excerpt/.
28. Friedman LD. (Personal Communication, May 3, 2022).
29. Gallo C, Ridolfi L, Scarsoglio S. Cardiovascular deconditioning during long-term spaceflight through multiscale modeling. NPJ Microgravity. 2020; 6(1):27.
30. Gertz ML, Chin CR, Tomoiaga D, MacKay M, Chang C, et al. Multi-omic, single-cell, and biochemical profiles of astronauts guide pharmacological strategies for returning to gravity. Cell Rep. 2020; 33(10):108429.
31. Hargens AR, Bhattacharya R, Schneider SM. Space physiology VI: exercise, artificial gravity, and countermeasure development for prolonged space flight. Eur J Appl Physiol. 2013; 113(9):2183–2192.
32. Heppenheimer TA. Colonies in Space. New York (NY): Warner; 1977.
33. Hodkinson PD, Anderton RA, Posselt BN, Fong KJ. An overview of space medicine. Br J Anaesth. 2017; 119(Suppl. 1):I143–I153.
34. Hughson RL, Helm A, Durante M. Heart in space: effect of the extraterrestrial environment on the cardiovascular system. Nat Rev Cardiol. 2018; 15(3):167–180.
35. Ikeuchi T, Sasaki S, Umemoto Y, Kubota Y, Kubota H, et al. Human sperm motility in a microgravity environment. Reprod Med Biol. 2005; 4(2): 161–168.
36. ISU MSS15 Team Project - Astra Planeta. 2015. [Accessed March 28, 2023]. Available from https://www.youtube.com/watch?v=qvMTTsbhKP4.
37. Iwase S, Nishimura N, Mano T. Osteoporosis in Spaceflight. In: Valds-Flores M, editor. Topics in Osteoporosis. London (UK): InTech;2013.
38. Jirak P, Mirna M, Rezar R, Motloch LJ, Lichtenauer M, et al. How spaceflight challenges human cardiovascular health. Eur J Prev Cardiol. 2022; 29(10):1399–1411.
39. Johnson RD, Holbrow C, editors. NASA SP-413 —Space Settlements—A Design Study, US Government Printing Office, Washington (DC); 1977.
40. Jones H. The partial gravity of the moon and mars appears insufficient to maintain human health. 50th International Conference on Environmental Systems (ICES) 2021. July 12–15, 2021; Federal Way (WA); 142.
41. Jonscher KR, Alfonso-Garcia A, Suhalim JL, Orlicky DJ, Potma EO, et al. Spaceflight activates lipotoxic pathways in mouse liver. PLoS One. 2016; 11(4):e0152877. Erratum: Spaceflight activates lipotoxic pathways in mouse liver. PLoS ONE. 2016; 11(5):e0155282.
42. Kao FC, Chiu PY, Tsai TT, Lin ZH. The application of nanogenerators and piezoelectricity in osteogenesis. Sci Technol Adv Mater. 2019; 20(1): 1103–1117.
43. Khaksarighiri S, Guo J, Wimmer-Schweingruber R, Narici L. An easy-to-use function to assess deep space radiation in human brains. Sci Rep. 2021; 11(1):11687.
44. Khine HW, Steding-Ehrenborg K, Hastings JL, Kowal J, Daniels JD, et al. Effects of prolonged spaceflight on atrial size, atrial electrophysiology, and risk of atrial fibrillation. Circ Arrhythm Electrophysiol. 2018; 11(5):e005959.
45. Kim DS, Vaquer S, Mazzolai L, Roberts LN, Pavela J, et al. The effect of microgravity on the human venous system and blood coagulation: a systematic review. Exp Physiol. 2021; 106(5):1149–1158.
46. Kwok AT, Mohamed NS, Plate JF, Yammani RR, Rosas S, et al. Spaceflight and hind limb unloading induces an arthritic phenotype in knee articular cartilage and menisci of rodents. Sci Rep. 2021; 11(1):10469.
47. Kwok AT, Moore JE, Rosas S, Kerr BA, Andrews RN, et al. Knee and hip joint cartilage damage from combined spaceflight hazards of low-dose radiation less than 1 G_y and prolonged hindlimb unloading. Radiat Res. 2019; 191(6):497–506.
48. Lackner JR, DiZio PA. Adaptation to rotating artificial gravity environments. J Vestib Res. 2003; 13(4–6):321–330.
49. Lackner JR, DiZio P. Space motion sickness. Exp Brain Res. 2006; 175(3):377–399.
50. Lee AG, Mader TH, Gibson CR, Tarver W, Rabiei P, et al. Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. NPJ Microgravity. 2020; 6(7). Erratum: Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. NPJ Microgravity. 2020; 6(23).
51. Lewis ML, Reynolds JL, Cubano LA, Hatton JP, Lawless BD, Piepmeier EH. Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J. 1998; 12(11):1007–1018.
52. Liakopoulos V, Leivaditis K, Eleftheriadis T, Dombros N. The kidney in space. Int Urol Nephrol. 2012; 44(6):1893–1901.

53. Liang P, Xu Y, Zhang X, Ding C, Huang R, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015; 6(5): 363–372.
54. Limper U, Tank J, Ahnert T, Maegele M, Grottke O, et al. The thrombotic risk of spaceflight: has a serious problem been overlooked for more than half of a century? Eur Heart J. 2021; 42(1):97–100.
55. Longnecker DE, Manning FJ, Worth MH Jr, editors. Review of NASA's longitudinal study of astronaut health. Washington (DC): National Academies Press; 2004.
56. Louis F, Deroanne C, Nusgens B, Vico L, Guignandon A. RhoGTPases as key players in mammalian cell adaptation to microgravity. BioMed Res Int. 2015; 2015:747693.
57. Madzunkov SM, Nikolić D, Belousov A, Fry D, Barzilla J, et al. Development of an active shielding concept using electrostatic fields. 50th International Conference on Environmental Systems (ICES) 2021. July 12–15, 2021; Federal Way (WA); 121.
58. Markley LC, Lindner JF. Artificial gravity field. Results in Physics. 2013; 3:24-29.
59. Marshall-Goebel K, Laurie SS, Alferova IV, Arbeille P, Auñón-Chancellor SM, et al. Assessment of jugular venous blood flow stasis and thrombosis during spaceflight. JAMA Netw Open. 2019; 2(11):e1915011.
60. Meerman M, Bracco Gartner TC, Buikema JW, Wu SM, Siddiqi S, et al. Myocardial disease and long-distance space travel: solving the radiation problem. Front Cardiovasc Med. 2021; 8:631985.
61. Merrill AH Jr., Wang E, Jones DP, Hargrove JL. Hepatic function in rats after spaceflight: effects on lipids, glycogen, and enzymes. Am J Physiol. 1987; 252(2):R222–R226.
62. Michel JB. Phylogenic determinants of cardiovascular frailty, focus on hemodynamics and arterial smooth muscle cells. Physiol Rev. 2020; 100(4):1779–1837.
63. Milojevic T, Weckwerth W. Molecular mechanisms of microbial survivability in outer space: a systems biology approach. Front Microbiol. 2020; 11:923.
64. Mishra B, Luderer U. Reproductive hazards of space travel in women and men. Nat Rev Endocrinol. 2019; 15(12):713–730. Erratum: eproductive hazards of space travel in women and men. Nat Rev Endocrinol. 2019; 15:744.
65. Mohammadyari P, Gadda G, Taibi A. Modelling physiology of haemodynamic adaptation in short-term microgravity exposure and orthostatic stress on Earth. Sci Rep. 2021; 11(1):4672.
66. Montesinos CA, Khalid R, Cristea O, Greenberger JS, Epperly MW, et al. Space radiation protection countermeasures in microgravity and planetary exploration. Life (Basel). 2021; 11(8):829.
67. Morey-Holton, E. The impact of gravity on life. In: Rothschild LJ, Lister AM, editors. Evolution on planet Earth: the impact of the physical environment. Cambridge (MA): Academic Press; 2003:143–159.
68. Obrador E, Salvador R, Villaescusa JI, Soriano JM, Estrela JM, Montoro A. Radioprotection and radiomitigation: from the bench to clinical practice. Biomedicines. 2020; 8(11):461.
69. National Research Council. Recapturing a future for space exploration: life and physical sciences research for a new era. Washington (DC): The National Academies Press; 2011.
70. Navasiolava N, Yuan M, Murphy R, Robin A, Coupé M, et al. Vascular and microvascular dysfunction induced by microgravity and its analogs in humans: mechanisms and countermeasures. Front Physiol. 2020;11:952.
71. O'Neill GK. The High Frontier—Human Colonies in Space. New York (NY): William Morrow & Co.; 1976.
72. Onorato G, Di Schiavi E, Di Cunto F. Understanding the effects of deep space radiation on nervous system: the role of genetically tractable experimental models. Front Phys. 2020; 8:362.
73. Ozdemir RA, Goel R. Critical role of somatosensation in postural control following spaceflight: vestibularly deficient astronauts are not able to maintain upright stance during compromised somatosensation. Front Physiol. 2018; 9:1680.
74. Patel ZS, Brunstetter TJ, Tarver WJ, Whitmire AM, Zwart SR, et al. Red risks for a journey to the red planet: the highest priority human health risks for a mission to Mars. NPJ Microgravity. 2020; 6(1):33.
75. Paul JO, Funmilola OA, Ayegba AS. Microgravity and infertility. IJTSRD. 2019 [Accessed on March 28, 2023]. 3(6):190–195. Available from https://www.ijtsrd.com/papers/ijtsrd29408.pdf.
76. Pavlakou P, Dounousi E, Roumeliotis S, Eleftheriadis T, Liakopoulos V. Oxidative stress and the kidney in the space environment. Int J Mol Sci. 2018; 19(10):3176.
77. Prisk GK. Microgravity and the lung. J Appl Physiol (1985). 2000; 89(1):385–396.
78. Puspitasari A, Cerri M, Takahashi A, Yoshida Y, Hanamura K, Tinganelli W. Hibernation as a tool for radiation protection in space exploration. Life (Basel). 2021; 11(1):54.
79. Ramachandran V, Wang R, Ramachandran SS, Ahmed AS, Phan K, Antonsen EL. Effects of spaceflight on cartilage: implications on spinal physiology. J Spine Surg. 2018; 4(2):433–445.
80. Roy-O'Reilly M, Mulavara A, Williams T. Review of alterations to the brain during spaceflight and the potential relevance to crew in long-duration space exploration. NPJ Microgravity. 2021; 7(1):5.
81. Siddiqui R, Akbar N, Khan NA. Gut microbiome and human health under the space environment. J Appl Microbiol. 2021; 130(1):14–24.
82. Smith JK. IL-6 and the dysregulation of immune, bone, muscle, and metabolic homeostasis during spaceflight. NPJ Microgravity. 2018; 4(1):24.
83. Smith SM, Heer MA, Shackelford LC, 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(9):1896–1906.
84. Stahl-Rommel S, Jain M, Nguyen HN, Arnold RR, Auñón-Chancellor SM, et al. Real-time culture-independent microbial profiling onboard the International Space Station using nanopore sequencing. Genes (Basel). 2021; 12(1):106.
85. Sun Y, Kuang Y, Zuo Z. The emerging role of macrophages in immune system dysfunction under real and simulated microgravity conditions. Int J Mol Sci. 2021; 22(5):2333.
86. Tash JS, Kim SH, Schuber M, Seibt D, Kinsey WH. Fertilization of sea urchin eggs and sperm motility are negatively impacted under low hypergravitational forces significant to space flight. Biol Reprod. 2001; 65(4): 1224–1231.
87. Tauber S, Lauber BA, Paulsen K, Layer LE, Lehmann M, et al. Cytoskeletal stability and metabolic alterations in primary human macrophages in long-term microgravity. PLoS One. 2017; 12(4):e0175599.
88. Tays GD, Hupfeld KE, McGregor HR, Salazar AP, De Dios YE, et al. The effects of long duration spaceflight on sensorimotor control and cognition. Front Neural Circuits. 2021; 15:723504.
89. Tischler ME, Slentz M. Impact of weightlessness on muscle function. ASGSB Bull. 1995; 8(2):73–81.
90. Trudel G, Shahin N, Ramsay T, Laneuville O, Louati H. Hemolysis contributes to anemia during long-duration space flight. Nat Med. 2022; 28(1):59–62.
91. Trudel G, Shafer J, Laneuville O, Ramsay T. Characterizing the effect of exposure to microgravity on anemia: more space is worse. Am J Hematol. 2020; 95(3):267–273.
92. Turroni S, Magnani M, KC P, Lesnik P, Vidal H, Heer M. Gut microbiome and space travelers' health: state of the art and possible pro/prebiotic strategies for long-term space missions. Front Physiol. 2020; 11:553929.
93. Vasin MV, Ushakov IB. Radiomodulators as agents of biological protection against oxidative stress under the influence of ionizing radiation. Biol Bull Rev. 2020; 10(4):251–265.
94. Vernice NA, Meydan C, et al. Long-term spaceflight and the cardiovascular system. Precis Clin Med. 2020; 3(4):284–291.
95. Vinogradova OL, Tomilovskaya ES, Kozlovskaya IB. Gravity as a factor in evolutionary adaptation of animals to living on the Earth. Hum Physiol. 2021; 47(7):716–734.
96. Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, et al. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet. 2000; 355(9215): 1607–1611.

97. Vico L, Hargens A. Skeletal changes during and after spaceflight. Nat Rev Rheumatol. 2018; 14(4):229–245.
98. Voorhies AA, Ott CM, Mehta S, Pierson DL, Crucian BE, et al. Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. Sci Rep. 2019; 9(1):9911.
99. Wassersug RJ. Vertebrate biology in microgravity. Am Sci. 2001; 89(1): 46–53.
100. Widrick JJ, Knuth ST, Norenberg KM, Romatowski JG, Bain JL, et al. Effect of a 17 day spaceflight on contractile properties of human soleus muscle fibres. J Physiol. 1999; 516 (Pt. 3):915–930.
101. Wiener TC. Space obstructive syndrome: intracranial hypertension, intraocular pressure, and papilledema in space. Aviat Space Environ Med. 2012; 83(1):64–66.
102. Willey JS, Kwok AT, Moore JE, Payne V, Lindburg CA, et al. Spaceflight-relevant challenges of radiation and/or reduced weight bearing cause arthritic responses in knee articular cartilage. Radiat Res. 2016; 186(4):333–344.
103. Winkler LH. Space colonization—some physiological perspectives. Aviat Space Environ Med. 1978; 49(7):898–901.
104. Winkler LH. Across the void—the Anderson correspondence. In: Fire beyond the darkness: a metaphysical journey. Victoria (Canada): First Choice Books; 2016.
105. Wu XT, Yang X, Tian R, Li YH, Wang CY, et al. Cells respond to space microgravity through cytoskeleton reorganization. FASEB J. 2022; 36(2):e22114.
106. Wuest SL, Gantenbein B, Ille F, Egli M. Electrophysiological experiments in microgravity: lessons learned and future challenges. NPJ Microgravity. 2018; 4:7.
107. Yang JQ, Jiang N, Li ZP, Guo S, Chen ZY, et al. The effects of microgravity on the digestive system and the new insights it brings to the life sciences. Life Sci Space Res (Amst). 2020; 27:74–82.
108. Yang JW, Song QY, Zhang MX, Ai JL, Wang F, et al. Spaceflight-associated neuro-ocular syndrome: a review of potential pathogenesis and intervention. Int J Ophthalmol. 2022; 15(2):336–341.
109. Young LR, Sutton JP, editors. Handbook of Bioastronautics. Berlin (Germany): Springer Nature; 2021.
110. Zhang Y, Wang Y, Li X, Zhang J, Mao J, et al. The LRP5 high-bone-mass G171V mutation disrupts LRP5 interaction with Mesd. Mol Cell Biol. 2004; 24(11):4677–4684.