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Spaceflight-Induced Bone Tissue Changes that Affect Bone Quality and Increase Fracture Risk

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Abstract

Purpose of Review Bone mineral density and systemic factors are used to assess skeletal health in astronauts. Yet, even in a general population, these measures fail to accurately predict when any individual will fracture. This review considers how long-duration human spaceflight requires evaluation of additional bone structural and material quality measures that contribute to microgravity-induced skeletal fragility.

Recent Findings In both humans and small animal models following spaceflight, bone mass is compromised via reduced bone formation and elevated resorption levels. Concurrently, *bone structural quality* (e.g., trabecular microarchitecture) is diminished and the *quality of bone material* is reduced via impaired tissue mineralization, maturation, and maintenance (e.g., mediated by osteocytes).

Summary Bone structural and material quality are both affected by microgravity and may, together, jeopardize astronaut operational readiness and lead to increased fracture risk upon return to gravitational loading. Future studies need to directly evaluate how bone quality combines with diminished bone mass to influence bone strength and toughness (e.g., resistance to fracture). Bone quality assessment promises to identify novel biomarkers and therapeutic targets.

Keywords Bone · Architecture · Quality · BMD · Fracture · Microgravity · Spaceflight

Introduction

Questions have long persisted as to the human body's ability to maintain operational capabilities during spaceflight and health status upon return to normal gravitational loading (1 g) [1]. Predictions before the first spacecraft were launched were speculative; hypothesising that humans would suffer from ailments ranging from hallucinations to infectious illnesses [2, 3]. The physiological effects of disuse had been observed clinically; thus, the conclusion was made that bones would "soften" in spaceflight. Our community has since come to confirm this finding. It is now widely agreed that fracture risk is elevated with increasing spaceflight duration, yet many questions remain as to the exact contributors to skeletal fragility with reduced gravity exposure.

Bone strength and fragility are major concerns for human spaceflight. Bone loss onset occurs within days of unloading, yet the round trip to Mars will take a minimum of one calendar year. The National Aeronautics and Space Administration (NASA) Human Research Program (HRP) has developed a Roadmap to outline Risks that directly, or indirectly, influence critical physiological systems and tissues, including bone [4, 5] (Fig. 1 and Table 1). These risks outline research priorities for long-duration human spaceflight of which nearly one-fifth pertain to the musculoskeletal system. Musculoskeletal tissues are critical for astronauts to perform essential duties, many of which are physically demanding including routine maneuvers within spacecraft to hours-long spacewalks requiring

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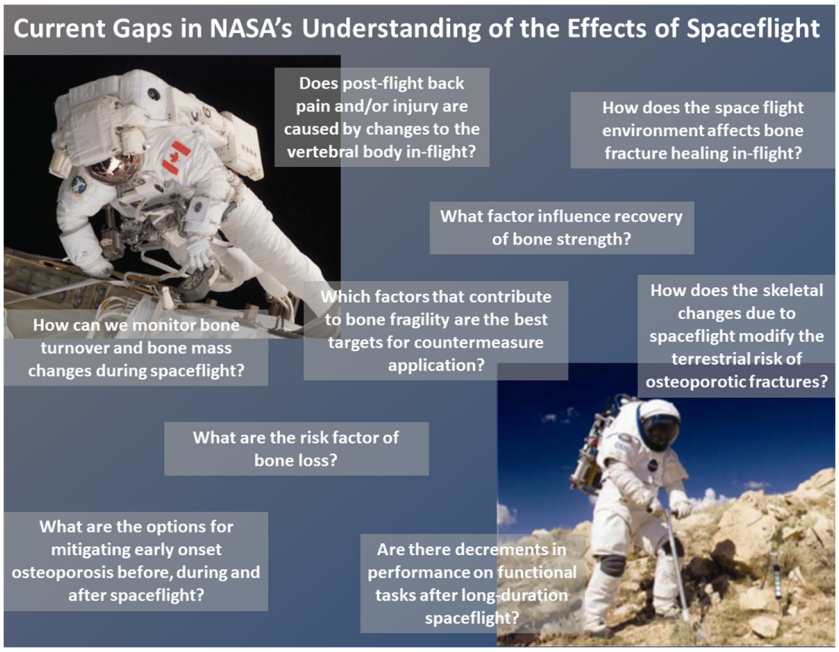

Fig. 1 Key Gaps in our knowledge, as identified by NASA's Human Research Program [4, 5], of how crew health and performance are influenced in and beyond low Earth orbit. These Gaps, and associated Risks, include both physiological effects as well as those that detrimentally affect performance from hazards such as radiation, altered gravity, and hostile environments. The Human Research Roadmap also identifies challenges unique to the space environment related to limited medical support, human factors, and behavioral health. Images used with permission from NASA Images

manipulation of both small tools and large, high-inertia objects [1]. Following spaceflight, astronauts are at greater risk of fracture due to bone loss [6] and falling associated with temporary or permanent changes to connective tissues [7] and vestibular [2, 8–10], vision [11], and central nervous system [12] impairments.

Despite substantive prior works, the complex mechanisms underlying spaceflight-induced bone loss and elevated fracture risk have yet to be elucidated. The clinical measure of bone mineral density (BMD) is the most common skeletal assessment performed on humans following spaceflight, yet accounts for only 60% of the variation in bone fragility [13]. Thus, the present review seeks to provide context into how spaceflight-induced bone changes are influenced by BMD *as well as quality of bone material* (Table S1). While exposure to radiation is harmful to bone, most spaceflight studies of humans and animal models to date have occurred in low Earth orbit and are largely protected from ionizing radiation [14]. Therefore, this review excludes consideration of radiation. Finally, a summary of key review articles that are central to human and rodent research following spaceflight is provided as an annotated bibliography (Table S2).

Bone Quality Is Critical for Fracture Prediction Following Spaceflight

NASA's research and astronaut bone health monitoring program seeks to collect data and identify occupational and health risks and knowledge gaps (Table 1 and Fig. 1). Non-invasive measurements of areal bone mineral density (aBMD) have been required of astronauts since ~ 2000 [6]. aBMD is collected during a clinical dual-energy X-ray absorptiometry (DXA) scan where bone mineral density is divided by bone area. aBMD is used to evaluate skeletal health in both the general population [15, 16] and in astronauts [6, 17••]. Similarly, volumetric bone mineral density (vBMD) is collected from quantitative computed tomography (QCT) [16]. When combined with urine and serum chemistry assessment to evaluate products of bone remodeling and associated factors [18], the predictive value of non-invasive bone metrics is increased. Yet, BMD and systemic bone biomarkers are indirect measures of skeletal integrity and fail to accurately identify which astronauts will experience fracture [19].

DXA scores are used to diagnose osteoporosis; however, only 54% of individuals who suffered hip fractures were classified as having osteoporotic BMD levels [20]. Bone strength and its resistance to fracture are dependent on the amount of bone tissue (i.e., BMD) *as well as* the quality of the bone material that is present [21–26] (Fig. 2). Bone quality is generally defined by a collection of architectural and material properties [27, 28] which are largely influenced by bone turnover, occur across multiple length scales, and contribute to structural rigidity [24, 26]. However, the quality of the bone material itself is equally important [26] and includes mineral and collagen properties, prevalence of non-collagenous proteins [29], tissue mineralization [30], cortical porosity [31], collagen crosslinking [32], and microdamage [27]. Further, mineral and collagen contain bound and unbound water, where a reduction in hydration may occur with spaceflight [33] and can lead to diminished bone strength and toughness [34, 35]. Many contributors to bone quality also serve as fracture toughening mechanisms [36]. Mineralized collagen fibrils promote strength and ductility, and crack-tip shielding occurs due to

Table 1 Selected Risks and Gaps from the NASA Human Research Roadmap that are most closely related to skeletal fragility and outcomes. A full list of Risks and Gaps are located here [5]. Note that some Risks are edited using "..." for brevity

Risk of early onset osteoporosis due to spaceflight—*Osteo*
Osteo 1. A new acceptable bone health standard using an expanded surrogate for bone health needs to be defined for the flight environment.
Osteo 2. What is the incidence & prevalence of early onset osteoporosis or fragility fractures due to exposure to spaceflight.
Osteo 3. We need a validated clinically relevant method for assessing the effect of spaceflight on osteoporosis or fracture risk in long-duration astronauts.
Osteo 4. We don't know the contribution of each risk factor on bone loss and recovery of bone strength, and which factors are the best targets for countermeasure application.
Osteo 5. We need an inflight capability to monitor bone turnover and bone mass changes during spaceflight.
Osteo 6. We need to identify options for mitigating early onset osteoporosis before, during, and after spaceflight.
Osteo 7. How do skeletal changes due to spaceflight modify the terrestrial risk of osteoporotic fractures?
Risk of bone fracture due to spaceflight-induced changes to bone—*Fracture*
Fracture 1. We don't understand how the space flight environment affects bone fracture healing in-flight.
Fracture 2. We need to characterize the loads applied to bone for standard in-mission activities.
Fracture 3. We need a validated method to estimate the risk of fracture by evaluating the ratio of applied loads to bone fracture loads for expected mechanically-loaded activities during a mission.
Risk of injury from dynamic loads—*Occupant protection*
OP-1. We do not understand the risk of injury associated with crewed vehicle landings and how this risk relates to the desired acceptable risk.
OP-2. We do not know how load dynamics and sex differences affect injury risk in spaceflight conditions and do not have adequate injury assessment reference values (IARVs) to mitigate the increased risk of injury to the crew.
OP-3. We do not have adequately validated analytical tools to inform design decisions or allow verification of vehicle occupant safety in impact conditions.
OP-4. We do not know the extent to which spaceflight deconditioning decreases injury tolerance for dynamic loads.
Risk of cardiovascular disease and other degenerative tissue effects from radiation exposure and secondary spaceflight stressors—*Degen*
Degen 1. How can tissue specific experimental models be developed for the major degenerative tissue risks... in order to estimate space radiation risks for degenerative diseases?
Degen 2. What are the adverse outcome pathways associated with degenerative tissues changes ...? What are the key events or hallmarks, their time sequence, and their associated biomarkers?
Degen 3. What are the progression rates and latency periods for radiation-induced degenerative diseases, and how do progression rates depend on age, sex, radiation type, or other physiological or environmental factors?
Degen 4. How does individual susceptibility... alter radiation-induced degenerative disease processes and risk estimates? Does individual susceptibility modify possible threshold doses for these processes in a significant way?
Degen 5. What quantitative procedures or theoretical models are needed to extrapolate molecular, cellular, animal results, or clinical human data to predict degenerative tissue risks in astronauts? ...
Degen 6. What are the most effective medical or dietary countermeasures to mitigate degenerative tissue risks? Are there common pathways that will benefit multiple tissues?
Degen 7. Are there synergistic effects from other spaceflight factors (e.g., altered gravity (μ-gravity), stress, altered immune function...) that modify space radiation-induced degenerative diseases in a clinically significant manner?
Concern of intervertebral disc damage upon and immediately after re-exposure to gravity—*IVD*
IVD 1. Determine whether post-flight back pain and/or injury are caused by changes to the vertebral body in-flight.
Risk of renal stone formation—*Renal*
Renal 1. What is the current state of knowledge regarding renal stone formation due to spaceflight?
Renal 2. What is the frequency of post-flight stone formation; the incidence and types of stones; and the time course of stone formation? How does tone formation correlate with food intake and hydration status?
Risk of inadequate nutrition—*Nutrition*
N 7.1. We need to identify the most important nutritional factors for musculoskeletal health.
N 13. Can renal stone risk be decreased using nutritional countermeasures?
Risk of adverse health event due to altered immune response—*Immune*
Immune 1. We do not know the influence, direct, or synergistic, on the immune system of other physiological changes associated with spaceflight.
Immune 2. We do not know the cumulative effects of chronic immune dysfunction on missions greater than six months.

extrinsic (e.g., osteons or collagen fibers that bridge emerging cracks) and intrinsic mechanisms (e.g., the collagen-mineral composite). Heterogeneity of bone structures and bone material imparts toughness by deflection of a crack's path, where too little or too great of variance enables unstable crack growth [36–38]. Heterogeneity in mineral crystal sizes and orientations, degree of mineralization, and the amount of collagen crosslinking also contribute to altered strength and tissue modulus [23, 27]. Finally, bone cells respond to dynamic mechanical and biological cues to form and maintain bone, where disruption of these signals leads to poor quality bone material. Fracture risk assessments should thus take into context BMD, bone material quality measures, and measurements of bone turnover to accurately assess fracture risk [30, 39].

Effects of Spaceflight on the Human Skeleton

Decrements to operational readiness and skeletal health are a significant challenge to long-duration human spaceflight.

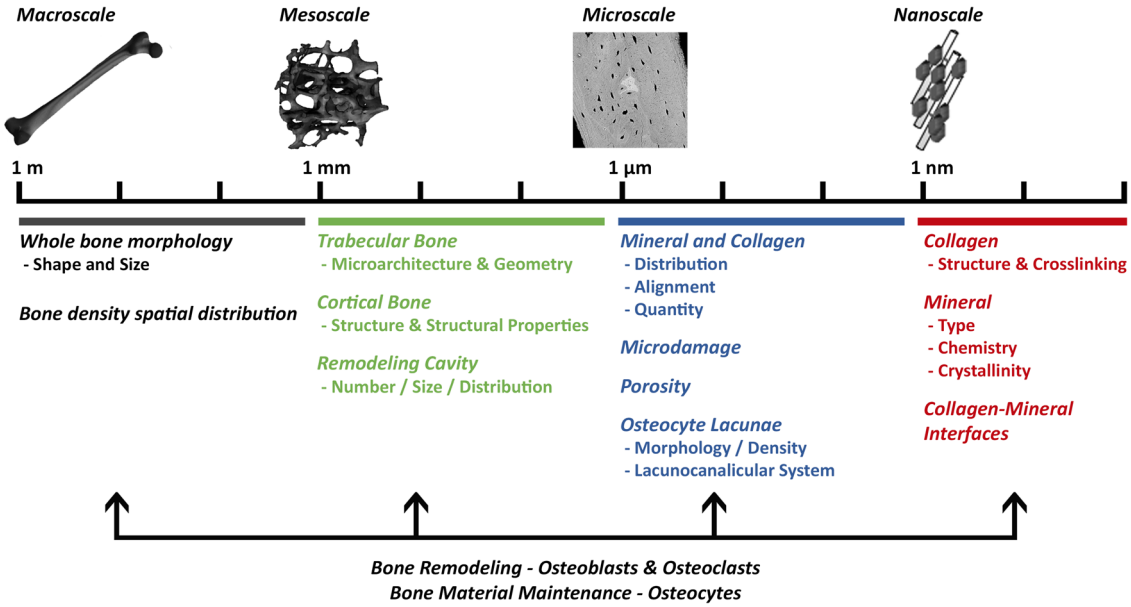

Fig. 2 Bone strength and toughness depend on bone mineral density (BMD) and a range of metrics that describe bone structural and material quality. All of these measures are controlled by bone modeling and remodeling, via osteoblasts and osteoclasts, as well as osteocyte-directed maintenance of local bone material. Aspects of bone material quality are shown on a scale depicting hierarchical structure of bone according to approximate length scales of analysis. Note: length scales were selected to match mechanical and compositional assessment techniques of bone quality as described by E. Donnelly in Methods for assessing bone quality: A review [28]

Seminal studies consistently show that elevated bone loss in weight-bearing regions of the human skeleton exceeds levels common in post-menopausal osteoporotic women (aBMD loss of 0.5–1%/year) [6, 40, 41]. Cosmonauts on the Russian Mir spacecraft for 4–14.4 months experienced aBMD losses of 1%/month from the spine and 1.5%/month from the hip, with pronounced bone loss at sites that are weight-bearing on Earth [40]. A subsequent QCT analysis of the hip of International Space Station (ISS) crewmembers replicated these results and revealed that bone loss occurred mainly due to cortical thinning: trabecular vBMD decreased by 0.4–0.5%/month and cortical bone by 2.2–2.7%/month [42].

Microgravity differently impacts bone loss in weight-bearing (tibia) and non-weight-bearing (radius) sites in cosmonauts, and local bone resorption is greater in trabecular than in cortical bone [41]. While the distal radius remained unchanged after 6 months in microgravity, vBMD in tibial trabecular decreased by 5.43% and cortical bone by 1.78%, and these losses were not recovered after 6 months in 1 g. Similarly, astronauts and cosmonauts returning from prolonged ISS flights failed to recover lost bone (i.e., hip trabecular BMD) after 2 years [17••]. Bone biomarkers in urine and serum follow DXA and QCT outcomes, where spaceflight is associated with diminished bone formation markers and elevated markers of bone resorption [43]. Sclerostin, a marker of osteocyte activity and inhibitor of osteoblast-mediated bone formation, increases with skeletal unloading [44–47] but remained unchanged in cosmonauts following 4–6 months on the ISS [48]. Countermeasures including bisphosphonates [6, 49–51] and exercise [43, 49, 52, 53] inconsistently prevent spaceflight-induced bone loss. Moreover, bisphosphonates have significant side effects and are not indicated for young, healthy adults due to microdamage accumulation and prolonged suppression of bone turnover [54]. And despite use of the advanced resistive exercise device (ARED), bone resorption markers in urine remained up to 3-fold higher than baseline and some ARED participants lost BMD at a rate of 1.5%/month [49, 53]. Significant gaps remain in our knowledge about skeletal adaptation and potential treatments to counteract the deleterious effects of reduced gravity.

Potential for Recovery Following Skeletal Unloading

Spaceflight has lasting negative impacts on bone strength, and yet no effective means of restoring bone mass have been identified. It can be argued that once a bony element, such as a trabeculae, is resorbed that no mechanical cues remain to signal osteoblasts to rebuild the structure. Predicting the fracture incidence of humans following spaceflight is complicated by a small population; thus, surrogate measures (e.g., trabecular microarchitecture from peripheral QCT) should be considered [6, 17••, 49]. While other physiological systems appear to reach a new equilibrium during prolonged spaceflight, bone loss may continue unabated, and recovery is slow and incomplete upon readaptation to 1 g [49, 55]. Indeed, BMD deficits persist after 4 years following prolonged ISS flights. Similarly, non-weight-bearing skeletal sites continued to deteriorate after 1 year of spaceflight and one additional year in 1 g [48]. When combined with aging in former astronauts, current strategies to

maintain skeletal structures and tissues may be insufficient to prevent fracture [56].

Complex Factors Are Involved in Human Spaceflight Studies of Bone

Our understanding of changes in bone material quality and bone strength in astronauts remains limited by the number of human subjects in space, lack of true ground analogs, inconsistent duration of exposure to reduced gravity, and non-invasive imaging tools and biochemical assessments for accurate fracture prediction. There also remains a lack of diversity. Of 550 crewmembers evaluated for skeletal changes, the majority were healthy, Caucasian, and male with a mean age of 38 years [57]. Moreover, access to tissue samples in human subjects is difficult. While several human bed rest studies have evaluated iliac crest biopsies, to our knowledge no studies have evaluated human bone biopsies following spaceflight. Therefore, animal (e.g., rat and mice) and other model systems (e.g., fish [43, 58]) are needed to understand skeletal adaptation to spaceflight and to evaluate potential therapeutic countermeasures.

Spaceflight and Simulated Microgravity Effects in Rats and Mice

From the range of animals flown since the early Apollo era, rats and mice have emerged as the most common organisms for the study of bone loss in space. Recent videos and behavioral assessment have shown that mice adapt well to microgravity and demonstrate species-typical behaviors [59]. However, rodent spaceflight habitat design varies widely; thus, environmental (e.g., habitat design) and other stressors may profoundly influence a wide range of physiological outcomes [60, 61, 62•, 63–65]. While not perfect, rodents provide an adequate analog of human bone physiology and are ubiquitous in pre-clinical research. They possess a similar genetic makeup as compared to humans [66], and their genomes can be altered for targeted studies. Rodents facilitate study of therapeutics, surgical interventions, and evaluation of the influence sex, age, and genetics. Potentially most beneficial is the ability to evaluate tissues from a large number of physiological systems. From a recent spaceflight experiments in 2018–2019, our group sampled > 95 tissues from each mouse spanning cardiovascular, neural, digestive, reproductive, immune, musculoskeletal, and other systems. With limited availability of tissues and spaceflight experiments, correlative analysis of existing [67] and novel data sets are important to drive new discoveries.

Bone Turnover is Affected by Microgravity Exposure

Astronauts exhibit systemically disrupted bone mineral homeostasis and bone loss that results from uncoupling of osteoblasts and osteoclasts during spaceflight [42, 43, 68, 69]. Similar outcomes have been observed in multiple studies of rodents on the Space Shuttle and ISS, where systemic measures and direct bone tissue assessments also point to disrupted bone formation and resorption. Bone formation is observed to decrease in most spaceflight studies of *growing* rodents while bone resorption is increased [43, 70–77]. Taken together, studies point to a common process underlying bone loss in both humans and rodents in spaceflight.

The shift towards bone catabolism with gravitational unloading has been commonly observed in growing rodents. Exposing young rats to microgravity for only 1 week resulted in measurable bone resorption and diminished mechanical properties [70, 77]. Growth plate abnormalities were noted despite normal longitudinal bone growth [71] and cortical thinning was caused by reduced periosteal apposition [72, 73, 76]. In 5-week-old rats flown for 10 days, expression of bone matrix protein-related genes and histological measures of bone formation were reduced [75, 78]. Rats aged ~ 4.5 weeks flown on the Space Shuttle (Space Transportation System) STS-77 mission (~ 2-week duration) showed site-specific reductions in bone formation when comparing across three long bones (i.e., humerus, femur, and tibia) on both periosteal and endocortical surfaces [79]. Osteoclast numbers and activity were also elevated along with reduced bone formation and osteoblast numbers.

Following earlier rat spaceflight investigations, murine spaceflight experiments have largely utilized growing mice. Developmentally, both male and female mice show histomorphometric evidence that periosteal bone formation ceases after 16–22 weeks of age [80, 81] and long bone mechanoresponsiveness is greatest at 4 months [82]. These ages are generalized and may differ across mouse strains [80–83]. The mouse skeleton achieves a fully grown, mature state around 4–6 months of age. Studies of spaceflight effects on growing mouse bone are attractive for the enhanced sensitivity to bone formation changes. For example, bone formation was markedly reduced, resorption increased, and the two processes appeared to be uncoupled in 9-week-old female mice on STS-108 (~ 13 days) [74]. Administering osteoprotegerin (OPG) to subgroups of mice from this study suppressed elevated bone resorption levels observed following spaceflight. Untreated flight mice showed reduced expression of bone formation biomarkers (e.g., osteocalcin), lower trabecular bone volume fraction (BV/TV), and diminished cortical periosteal bone formation as compared to ground controls. Additionally, this work showed increased trabecular bone surfaces occupied by osteoclasts and a non-significant reduction of osteoblast surfaces. Several additional mouse experiments on the ISS launched 8–9-week-old, male mice [84–86], including one of the longest flight experiments (91 days) to date which demonstrated greater effects in bones that were load-bearing on Earth [84, 85]. However, this study was limited by housing mice singly and small animal numbers

(i.e., only one wild-type and two transgenic mice survived). Finally, 16-week-old mice flown on STS-131 (15 days) [87], an age where fluorescent-labelling of bone formation is still measurable [80], showed reduced pelvic bone volume and thickness following spaceflight with no concomitant reduction of BMD. Compared to ground controls, flight mice revealed greater numbers of TRAP-positive osteoclasts and increased osteolysis surrounding osteocytes which expressed both the osteoclast marker TRAP and the matrix-degrading enzyme MMP10. Downregulation of p53, which is involved in the cell cycle, and upregulation of CDKN1a/p21, a cell cycle arrest mediator, indicated diminished osteogenesis and bone formation. These changes may be mediated through altered Wnt signaling, where SOST mRNA expression was significantly (16-fold) greater in spaceflight mice, thus indicating a potential mechanism underlying the suppression of bone formation [88].

Microgravity acts through early mesenchymal and hematopoietic stem cell differentiation to be a potent inhibitor of tissue growth and regenerative potential [89•]. Spaceflight redirects differentiation pathways for mesenchymal stem cells from osteoblasts to adipocytes [90, 91]. Osteoblasts exposed to microgravity or simulated microgravity undergo cytoskeletal changes [92–94] along with altered focal adhesions [95, 96], matrix proteins [94], interactions between cytokines and cytokine receptors [96–98], and inhibition of protein signals required for osteoblast differentiation [99]. Notably, rat osteoblasts cultured for 4–5 days in microgravity showed reduced mRNA levels for osteopontin and alpha-tubulin and increased the cell matrix receptor CD44 [94]. Additionally, osteoblast-specific transcription factors were decreased in simulated microgravity [99, 100]. Following spaceflight, human osteoblasts retain aberrant morphology, disorganized microtubular networks, and redistributed organelles [101]. Studies of osteoclast cultures in spaceflight are uncommon [102], yet autophagy may enhance osteoclast differentiation and osteoclastogenesis in simulated microgravity [103]. Looking to other organisms, medaka fish reared on the ISS demonstrated increased osteoclast activation along with reduced bone and tooth mineralization [58].

More recent studies of mice have employed older mice that may better predict bone physiological outcomes observed in adult humans. On the unmanned Bion-M1 biosatellite mission, mature (23-week-old at launch) male C57BL6 mice were flown for 30 days (high-orbit spaceflight environment) followed by 8 days in 1 g. This treatment resulted in a striking reduction of both axial and appendicular skeletal bone with cortical thinning and reduced trabecular connectivity and thickness [104•]. These changes were attributed to increased osteoclast surfaces and reduced osteoblast surfaces in femur, but not trabecular bone in the lumbar vertebrae. Notably, recovery increased osteoblast surfaces and decreased osteoclast surfaces in lumbar vertebrae. Mineral and collagen within bone tissue were preserved, yet tissue modulus (from nanoindentation) was reduced at some sites. Osteocytes were smaller, rounder, and underwent increased apoptosis [104] in contrast to osteocytic osteolysis that was observed in two separate studies of 16-week-old C56BL/6 mice in microgravity (females) [87] and simulated microgravity (males) [105]. These latter observations motivate systematic evaluation with varying factors including age of microgravity onset, duration of unloading, sex, and genetics.

NASA Rodent Research (RR) missions that commenced after retirement of the Space Shuttle provide improved access to tissues from post-adolescent mice, and hold potential to guide future investigations. The RR studies to date have included mice (aged ≥ 12 weeks [80, 106]) of two strains: C57BL/6 (e.g., RR-1, 2) and BALB/C (e.g., RR-3, 5, 8), and include multiple age groups within a single flight (e.g., RR-8 with 10- and 31-week-old at launch). For example, we compared trabecular microarchitecture from female C57Bl/6 flight mice aged 9- and 32-week-old at launch, termed as Young and Mature, that were flown on STS-118 and the RR-1 SpaceX-4 missions for 12.8 and 21 days, respectively [107]. When normalizing data by the number of days flown, trabecular bone changes were dominated by diminished formation in Young mice and elevated resorption in the Mature cohort. These preliminary data indicate that mature bone may be at greater risk of deleterious microarchitectural changes from disuse than growing bone. Also similar to humans and rats, greater bone loss was observed in the femur than in the vertebrae *thus deleteriously affecting the quality of load-bearing bone structures*.

Spaceflight-Induced Architectural Changes in Bone Span Multiple Length Scales

Osteocytes are central to the maintenance of extant and newly formed bone material. These cells form roughly 95% of the bone cell population [108] and direct local maintenance of bone material quality through local bone resorption and deposition in response to endocrine [109] and mechanical stimuli [105]. Osteocyte lacunae in the iliac crest of monkeys flown on Bion-11 were infilled with collagen fibrils, indicating apoptosis, and showed increased perilacunar resorption [110]. These results are compelling, but inconsistent results from prior studies indicate that osteocyte responsiveness may depend on age [85, 87, 104]. We used nanocomputed tomography (voxels = 0.6 μm) to evaluate osteocyte lacunar morphology [111, 112] within mid-tibial cortical bone from the aforementioned Young (STS-118) and Mature (RR-1) mice [113]. Following spaceflight, lacunae from Young mice became less elongated and more spherical, a shape that is consistent with an aging osteocyte phenotype and likely possesses diminished strain sensitivity [108, 109, 114]. While lacunae in Mature mice underwent no shape change, lacunar volume increased.

This is of concern for crewmembers who return to Earth as bone material changes occur slowly and spherical sensors are less able to detect the magnitude and direction of a tensor such as strain.

Collectively, these results demonstrate that osteocytes may be irreparably affected by disuse in microgravity and may deleteriously affect the quality of the surrounding extant bone over the long-term. Microgravity exposure may thus influence osteocytes to contribute to poor *bone material quality, impaired bone formation, bone resorption, reduced recovery, and consequential bone fragility.*

The Material Composition of Bone Is Altered with Microgravity Exposure

Similar spaceflight effects on BMD and bone *structural* quality, evidence also demonstrates a pronounced influence on bone *material* quality. Growing rats on the unmanned Cosmos 1887 biosatellite (12.5 days) produced the most thorough assessment of bone material quality changes to date. With microgravity, the strength and stiffness of the L6 vertebrae were significantly diminished [115]. Vertebrae and calvaria had low percentages of phosphorous and magnesium, and increased sulfur, indicating slow maturation of newly formed bone [116]. Mineralization profiles within calvaria had lower density fractions than ground controls and a smaller hydroxyapatite crystal c-axis [117]. Spaceflight was associated with a steeper gradient of decreasing mineral and osteocalcin concentration towards the distal femoral diaphysis and a lower collagen concentration (i.e., indicating suppressed formation or accelerated collagen degradation) toward the proximal diaphysis [118]. A smaller proportion of mature hydroxyproline crosslinks with spaceflight indicated impaired mineralization [115, 119]; crosslinks provide nucleation sites for the bone apatite nucleation and crystal growth [117]. These results indicate that bone material formed in microgravity possesses inferior material quality. Similarly, in 8-week-old rats on Spacelab-3 (7 days) [118], both vertebrae and humeri possessed reduced bone mineral and osteocalcin, increased hydroxyproline concentration, and reduced phosphorus. The phosphorus reduction was hypothesized to account for reduced bone strength [118] as well as for mineralization defects in non-weight-bearing bones [120, 121]. In this same experiment, decreased bone growth and turnover occurred concomitant with smaller mineral apatite crystal size [117]. Finally, in 56-day-old male rats on NASA Spacelab (STS58-SLS2, 14 days), reduced BMD was accompanied by lower calcium and phosphorus concentrations in the proximal tibia [77]. Mineral composition of trabecular bone indicated brushite, which is a precursor of hydroxyapatite [122], suggesting that spaceflight inhibited or delayed mineral maturation.

To our knowledge, only one mouse spaceflight experiment has assessed bone material quality. In 23-week-old mice on Bion-M1 (30 days) [104•], nanoindentation modulus, hardness, and energy dissipation of the L1 vertebrae were unchanged with spaceflight. However, nanoindentation of femur cortical bone revealed diminished modulus and hardness. The degree of mineralization, crystallinity index, mineral maturity, carbonation, and collagen maturity remained unchanged indicating that the mineral quality was preserved despite skeletal unloading. This study reaffirms that in the unique Bion-M1 environment [62], microgravity differentially affects load and non-load-bearing bones differently but fails to capture compositional changes in prior studies of growing rat bone [77, 117, 121]. Bone material quality has been definitively linked to strength and toughness, and mechanical property deficits are observed following microgravity exposure [74, 76, 79, 123], yet again the influence of factors such as sex, age, genetics, duration of microgravity exposure, and stress is critical to ultimately evaluate risks for fragility fractures in astronauts.

Spaceflight Effects May Promote an Accelerated Aging Phenotype that Contributes to Poor Bone Quality

Substantive research to date suggests that the space environment promotes phenotypes typical of aging and diminished regenerative potential of a range of tissues [124]. The NASA Twins Study demonstrated telomere elongation, genome instability, cardiovascular dysfunction, ocular deficits, and DNA methylation changes in immune and oxidative stress-related pathways [11]. Fibroblasts flown on STS-93 exhibited gene expression profiles indicative of cellular stress signaling, which may induce cellular senescence and apoptosis [125]. Skeletal muscles from mice on Bion M1 underwent significant atrophy and possessed impaired compensatory muscle fiber regenerative processes [126]. Mesenchymal and hematopoietic cell differentiation capacity is downregulated after 15 days in microgravity thus diminishing regenerative potential [89]. Similar outcomes widely present following spaceflight including fatty liver disease that develops in adolescent mice [127] along with degeneration in the eye [11, 128, 129], brain [11, 130], immune system [11, 131, 132], and cardiovascular tissues [133, 134]. These changes collectively illustrate a systemic disruption of processes following spaceflight that also occur with aging.

The parallels between spaceflight and aging are also compelling for skeletal tissues [124]. Bone fragility, a hallmark of aging and of spaceflight, is associated with decreased bone formation and increased bone resorption: a net loss of trabecular microarchitecture, cortical thinning and diminished structural properties [27, 80, 106, 124, 135–137], and impaired cellular regenerative potential [89]. Furthermore, aging bone cells detrimentally alter the composition of the inorganic and organic components of bone resulting in higher mineralization, altered mineral chemistry and crystallization [27, 104•, 117, 118, 138–140], and decreased enzymatic to non-

enzymatic crosslinking in collagen [21, 140]. Microdamage also accumulates [135, 138, 140]. Thus, as bone tissue ages, and possibly following reduced gravitational loading, decreased cellular functionality facilitates deleterious changes to the bone material quality and increases fracture susceptibility.

Complex Factors Involved in Studies of Bone from Small Animal Models

Improvements are needed to better elucidate mechanisms underlying bone material quality changes and skeletal fragility in spaceflight. Animal studies currently need crew interaction, and crewmembers need specialized training to monitor and dissect animals in space. Crew time on orbit is limited, and animal experiments are time-consuming. Mice are an attractive model due to their small size, economy, and ease of genetic manipulation; however, their bones are not Haversian and a cephalic fluid shift is observed only in rats (not mice). The use of inbred strains excludes the genetic diversity in a human population. Most studies utilize a single sex, yet sex hormones are integral to bone mechanobiology [141, 142].

Moreover, measures of genes and other biological markers may reflect a lengthy return to Earth rather than being indicative of physiological conditions in space. The habitats of the animals are also complex: e.g., lack of suitable enrichment, single versus group housing, and environmental factors (e.g., noise, lighting) may contribute to stress. And it remains unclear how to compare results from varying rodent habitats with the differing environmental stressors that have occurred in the many existing spaceflight studies [60, 61, 62•, 63–65]. However, the most important limitation may be the lack of a true control. While ground-based analogs attempt to emulate caging and environmental conditions in space, gravity is always present on Earth and the 3D-spaceflight environment is difficult to recapitulate [43].

Conclusions and Looking Towards the Future

Recently, NASA announced goals through 2024 including placing the first woman on the Moon [143] and opening the ISS for commercial business [144]. Private astronauts could fly for up to 30 days starting in 2020 [144]. These initiatives stress the importance for continued investigation of the musculoskeletal system and create potential to better evaluate a more diverse, human population.

To make strides in spaceflight research, the capacity of spaceflight animal hardware needs to be increased to allow for different strains, sexes, and larger group sizes. These improvements would facilitate investigations of a wide range of single or multiple physiological systems as well as targeted questions such as *how bone material quality influences skeletal tissue fracture* and *are skeletal decrements sustained in microgravity*? These advances hold potential to truly advance our knowledge to ultimately preserve the human skeleton in space.

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Compliance with Ethical Standards

Conflict of Interest Jennifer Coulombe, Bhavya Senwar, and Virginia L. Ferguson declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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