Bone health in spacefaring rodents and primates: systematic review and meta-analysis

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1. Abstract

1.1 Abstract (English)

Animals in space exploration studies serve both as a model for human physiology and as a mean to understand the physiological effects of microgravity. Animal experiments provided several advantages over human studies as they allow for post-mortem analysis of experimental subjects and larger sample sizes making statistical significance easier to achieve. To quantify the microgravity-induced changes to bone health in animals, we systematically searched Medline, Embase, Web of Science, BIOSIS, and NASA Technical reports. We selected 40 papers focusing on the bone health of 95 rats, 61 mice and 9 resus monkeys from 22 space missions ranging from 4 to 39 days in duration. The percentage difference from ground control in rodents was –24.1% [Confidence interval: -43.4,-4.9] for trabecular bone volume fraction and –5.9% [-8.0, -3.8] for cortical area, suggesting that trabecular bone is more affected than cortical bone by spaceflight in rodents. In primates, trabecular bone volume fraction was lower by –25.2% [-35.6, -14.7] in spaceflight animals compared to GC. Bone formation indices in rodent trabecular and cortical bone were significantly lower in microgravity. In contrast, osteoclast numbers were not affected in rats, and were variably affected in mice. Thus, microgravity induces bone deficits in rodents and primates likely through the suppression of bone formation.

1.2 Résumé (French)

L’utilisation des animaux dans les études d'exploration spatiale servent à la fois de modèle pour la physiologie humaine et de moyen afin de comprendre les effets physiologiques de la microgravité. Les expériences sur les animaux ont fourni plusieurs avantages par rapport aux études sur l'homme, car elles permettent une analyse post-mortem de sujets expérimentaux et des échantillons de plus grande taille, ce qui facilite l'obtention d'une signification statistique. Pour quantifier les changements induits par la microgravité dans la santé des os chez les animaux, nous avons systématiquement effectué des recherches dans les rapports techniques Medline, Embase, Web of Science, BIOSIS et NASA. Nous avons sélectionné 40 articles portant sur la santé osseuse de 95 rats, 61 souris, et 9 singes resus, sur 22 missions spatiales d'une durée de 4 à 39 jours. La différence en pourcentage par rapport au contrôle au sol chez les rongeurs était de –24,1% [Intervalle de confiance : -43,4, -4,9] pour la fraction volumique de l'os trabéculaire, et de –5,9% [-8,0, -3,8] pour l'aire corticale, suggérant que l'os trabéculaire est plus affecté que l'os cortical par le vol spatial. Chez les primates, la fraction volumique osseuse trabéculaire était inférieure de –25,2% [-35,6, -14,7] chez les animaux de vol spatial par rapport à la GC. Les indices de formation osseuse dans l'os trabéculaire et cortical des rongeurs étaient significativement plus faibles en microgravité. En revanche, le nombre d'ostéoclastes n'a pas été affecté chez les rats, et a été affecté de manière variable chez les souris. Ainsi, la microgravité induit des déficits osseux chez les rongeurs et les primates probablement par la suppression de la formation osseuse.

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3. Contribution of Authors

Matthew Goldsmith, Master of Science Candidate (co-primary author): performed screening, study characterization of the secondary search, data-extraction, critical appraisal and synthesis. Preparation of the final figures and manuscript.

Janet Fu (co-primary author): performed meta-analysis calculations.

Sequoia D. Crooks and Sean F. Condon: performed screening and study characterization of the primary search.

Martin Morris: developed the search strategy.

Svetlana V. Komarova: supervisor, performed title and abstract screening of secondary search, critical appraisal and synthesis and involved in manuscript preparation and editing.

4. Introduction and Objectives

4.1 Introduction

With plans by NASA to return humans to the lunar surface by 2024 [1] and to have the first ever astronauts journey to Mars within the next 2 decades [2], in addition to private interests in developing the first human colony on the Martian surface [3], human space travel will no doubt continue if not increase in the following century. Despite these high ambitions, we still do not fully understand the cause of physiological changes we observe in astronauts who travel to space, one of which is microgravity induced bone loss [4,5], however, the underlying cause of bone loss in astronauts is still largely unknown [4].

Animals have long been used as models to assess the physiological changes observed as a result of various stimuli and inform their impact to human health. Space-travelling animals have even preceded human, with several dogs, rodents, and primates being sent to space in the late 1940s - 1960s [6]. After developing the necessary technology allowing mammals to survive all phases of spaceflight, beginning in the 1970s animal experiments shifted to focus on the physiological effects of space travel [7]. The information obtained in animal studies significantly augmented our knowledge regarding human adaptations during space-travel. Experiments assessing skeletal changes in animals have the benefit of the collection bone biopsies, which is absent in astronaut studies. These biopsies have allowed for investigation into changes to cellular and molecular components of bone associated with microgravity, and thus provide further insight into the underlying mechanisms of microgravity induced changes in bone health. These missions however come at a considerable price, and it has been estimated that NASA spent $1.2 billion per launch over the period from 1982 to 2010 [8], therefore it is critically important to gain as much knowledge as possible from all the space experiments.

Even with the benefits of animal studies, and a significant expense associated with their execution, these experiments have not yet been used for the purposes of quantitative data synthesis. To overcome the problems associated with small sample sizes and high degree of variability between individual mission we employed meta-analysis to improves statistical power of all the studies.

4.2 Objectives

The objectives of this study were to (*i*) to systematically identify all the published literature regarding bone health in vertebrate animals that were part of experiments performed in space; (*ii*) use a meta-analytic approach to quantitatively characterize space-related changes to bone architecture and turnover in animals, (*ii*) identify cofounding variables associated with changes in bone health.

5. Review of the Literature

5.1 Overview of space exploration

Since the mid 20th century humans have been captivated by space travel. The early and rapid increase in the development and experiments of human space travel is often cited to be the result of the competition between the United States and the Soviet Union during the Cold War, using missile technology originally developed during World War II to accomplish this [9]. The first human sent to space was Yuri Gagarin who orbited the Earth on the Russian Vostok 1 on 1961. A mere 8 years later in 1969, Apollo 11 facilitated Neil Armstrong famous steps onto the Lunar surface [10]. Following the end of the Apollo lunar mission in 1972, all manned missions have been limited to low earth orbit [7], however are expected the have a resurgence with plans to return to the lunar surface [1], and finally have humans manned mission to Mars [2]. These missions to mars and further celestial surfaces have to overcome a number of challenges before they are possible. Apart from technological limitations, there are risks to human health associated with the required longer duration space missions [11]. These risks are a central concern in space medicine, a field intuitively defined by Williams and Turnock as "*the area of medical practice that deals with the provision of healthcare in partial and microgravitational environments"* [10].

5.2 Microgravity induced physiological changes in humans

Microgravity has been reported to have an effect on various aspects of human physiology. These risks to human health include but are not limited to: low venous pressure causing swelling of the upper body during the flight period [12], impairment of vision and swelling of the optical disc continuing post-flight [11,13], radiation induced illness and later carcinogenesis [4,11], and of particular importance for us, reduced quality of bone during and post-flight [4,5,11,13]. Although for several of these effects, countermeasures have been implemented to help mitigate or alleviate

them, the current countermeasures for microgravity induced bone loss, primarily exercise & diet [4,11], have not been completed effective [4]. We are limited by an incomplete understanding of the underlying cause of bone loss, which is required to develop more effective countermeasures [4,5].

5.3 History of animals in spaceflight

Animal space-travel preceded that of humans by more than a decade, with its early days being rather tragic. The first attempt to send an animal to space was on June 11th, 1948, when Albert I, a rhesus monkey aboard a V-2 Blossom, a repurposed WWII missile, was launched from New Mexico, United States. Sadly, Albert I suffocated during the mission, and the missile never reached the heights of space [6]. This was followed by numerous other failed attempts until November 3rd, 1957, when the Soviet Sputnik 2, an artificial earth satellite (AES), housing the now famous dog Laika managed to obtain orbit [6,14]. Laika was hooked up to an electrocardiogram, breath sensor, among several other sensory devices. After 5 hours of flight, the air temperature within Laika's cabin rose to 41°C, which is believed to be the cause of her death [14]. However, analysis of Laika's vitals during the early stages of the mission revealed that life can survive microgravity, and is believed to be a major catalyst for the first human orbital flight several years later [7,14]. The years that followed the Apollo lunar missions (post-1973) were dominated by space-experiments that focused on identifying and understanding the physiological changes that occur during microgravity, of which, animal studies became a primary focus [4,7]. Animal experiments provided several advantages over human studies in identifying these changes as they allow for post-mortem analysis of experimental subjects, factors such as diet and genetic variation to be accounted for, statistical significance easier to achieve with the larger sample sizes, and the short lifespan of the subjects allows us to extrapolate potential changes to humans during long duration missions [13].

5.4 Overview of the skeletal system and its health

The mammalian skeletal system serves several crucial functions including enabling movement, protecting the internal organs, and regulating the extracellular fluid given that bone is the main reservoir for calcium, phosphate and bicarbonate [15]. Pathological loss of bone, which may develop into osteoporosis, results in weaker bones more prone to fracture. These pathological fractures can significantly affect a person's mobility and often autonomy considering osteoporosis is most common in older aged individuals, especially post-menopausal women [16]. There are several methods to assess the health or quality of bone. Traditionally, determining bone mineral density using Dual-energy X-ray absorptiometry (DXA) [17], however more recently it has been argued that bone architecture is an equally important method of assessing bone health. The benefit of assessing bone architecture is the ability to identify changes to specific bone tissues, something standard DXA measurements cannot distinguish. Historically, assessing bone architecture was only possible via histomorphometry on bone biopsies but now can be done non-invasively with micro-computer tomography, μCT [18].

5.5 Division of the skeletal system & development

The skeleton can be divided into two major groups, (1) the appendicular skeleton: composed of bones of the upper and lower limbs, and (2) the axial skeleton: composed of bones of the skull, spine, thorax and pelvis [15]. Two mechanisms are responsible for the development of bone: endochondral ossification, the process by which most bone is formed, and intramembranous ossification. Both processes begin with mesenchymal progenitor cells. In the case of endochondral ossification, progenitor cells differentiate into chondrocytes (cartilage cells) which proliferate and act as a template to for later bone formation, while for intramembranous ossification, the progenitor cells differentiate directly into bone forming cells [19]. Intramembranous ossification is

only responsible for the development of flat bones in the skull, mandible and clavicles, while endochondral ossification is responsible the development of all other bones [19,20]. Additionally, endochondral ossification is responsible for longitudinal growth in long bones of the appendicular skeleton following birth [20]. In humans longitudinal bone growth in long bones continues until young adulthood. This is not the case for rodents where longitudinal bone growth continues past sexual maturity [21].

5.6 Composition of bone

There are three non-cellular components of bone: an organic component, a mineral component, and water. The organic component, also known as osteoid is made up of fibrous proteins, primarily Collagen Type I accounting for approximately 90% of its composition [19,22,23], also non-collagenous proteins and other minor types of collagen [22,24]. The mineral component is primarily composed of a calcium-phosphate-hydroxide salt called hydroxyapatite (HA), which forms crystals on the osteoid scaffold [22,24].

5.7 Bone tissues: cortical and trabecular

Within individual bones, we can identify two macroscopically distinct tissue types, cortical bone and trabecular bone. Although their compositions are similar, their architecture and in turn appearance are not.

Cortical bone, also known as compact bone, is characterized by low porosity and high density. Although cortical bone has a solid appearance, it has embedded pores which allows for a vascular network to supply nutrients to cells embedded in bone, osteocytes (we will return to these) [25]. The cortical bone confers the majority of bone's mechanical strength [15,25], and comprises the exterior surface of bone. Architectural parameters specific to cortical bone include cross-sectional

area of cortical bone, thickness of cortical bone, and in the case of long bone diaphysis (mid-shaft), the cross-sectional area of the bone marrow canal.

Cancellous bone, also referred to as trabecular bone or spongy bone due to its appearance, is highly porous with only 50-90% of the total volume being occupied by bone [25]. Trabecular bone is made of many single units known as trabeculae, small pieces of bone shaped as plates or rods [15]. Trabecular bone is much weaker compared to cortical bone, however, it does contribute to overall bone mechanical properties, acting as an internal support to strengthen bone [25]. It is often found on the interior of bones, within the cortices of flat bones and vertebrae, and in the epiphyses and metaphyses (the ends) of long bones. Trabecular bone within the metaphyses of long bones can be further subdivided into primary and secondary spongiosa. During longitudinal bone growth, primary spongiosa is formed first from organic matrix made by chondrocytes, and is later replaced with mature trabecular bone, secondary spongiosa [26]. Architectural parameters to determine health and in turn strength of trabecular bone include: trabecular bone volume to total volume fraction, average thickness of trabecular units, average length separating trabeculae, and average number of trabeculae per unit length.

5.8 Cells involved in bone turnover

The two primary effectors in bone turnover are osteoblasts and osteoclasts. Osteoblasts are the primary bone forming cell responsible for producing osteoid, the organic matrix of bone [15,19], and are involved in early bone mineralization via the production of membrane-bound matrix vesicles (MV). These MVs contain transporters and enzymes that concentrate calcium and phosphate which then precipitate to form HA crystals on the organic bone matrix [22,27]. Osteoclasts are considered the sole bone destroying or resorbing cell [28]. When active, osteoclasts tightly bond underlying mineralized bone creating a small pocket or microenvironment isolated

from the extracellular space. This microenvironment (a) is made highly acidic, leading to the dissolution of HA crystals, and (b) contain proteases, leading to degradation of the osteoid [15,28].

The third important cell type in bone is osteocytes, cells that are formed during the process of bone formation when some osteoblasts become embedded in the matrix [15]. Osteocytes are the most abundant cells in the bone, the primary function of which is to act as local sensors to changes in bone loading and to modulate bone formation or resorption by affecting osteoblast or osteoclast function [19,29,30].

5.9 Bone remodeling

In humans, bone mass only increases up until young adulthood after which bone growth ceases. During this period overall bone mass done not change, however bone does remains in a constant state of renewal via the process of bone remodeling. Bone remodeling refers to the process in which bone resorbing osteoclasts are coupled with bone forming osteoblasts in what is known as basic multicellular units (BMU) [16]. When the rate of bone formation matches the rate of bone resorption, bone is said to be in a state of homeostasis, when these rates become uncoupled, changes to bone mass occur [15,31]. Bone turnover is a surface dependent process. Considering the porous nature of trabecular bone, it has a much greater surface area to volume ratio, and as a result, it is renewed far more rapidly compared to cortical bone. Because of this, in a state of bone loss, representing a proportionally greater rate of bone resorption, loss to trabecular bone is greater [15].

6. Methods

This study was compliant with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement. Refer to Supplemental **Table S1** for PRISMA Checklist.

6.1 Search Strategy, Inclusion Criteria and Quality Assessment

A systemic search strategy using terms related to bone, space travel, and animals, including the names of individual missions, bones, and species of nonhuman vertebrates (Supplementary Information S1) was constructed by a medical librarian (MM). Medline, Embase, PubMed, BIOSIS Previews, and Web of Science were searched on November 2nd, 2017. An updated search was performed on November 1st, 2019. Additionally a manual search of the NASA Technical Report Server and articles referenced the compendium of animal and cell spaceflight experiments compiled by Ronca et al.[13] was performed. Studies in any language were considered. Title and abstract screening for the original search was performed independently by SDC and SFC, and for the update by SVK. Inclusion criteria was that the article describes any vertebrate species that was taken on a space mission. Studies describing invertebrate animals, humans, or Earth-based spaceflight simulations were excluded. After intermediate analysis, only studies describing spaceflight results for mice, rats and primates were included in full text screening for quantitative measurements related to bone health, which was performed by SDC, SFC and MG for the initial search and by SVK and MG for the update. In the final meta-analysis, we included the studies that presented quantitative measurements of trabecular and cortical architecture or bone turnover for bones of axial and appendicular skeleton excluding facial bones. Animals that were pregnant, or received surgery other than sham, abnormal diet, or hormone supplements, were excluded. Papers presenting average data without a measure of variation were excluded. Included papers were

scored for reporting quality (Supplementary Information S2), if two different species were reported in a single paper, they were scored independently.

6.2 Data extraction

For studies included after abstract/title screening, the year of publication, animal species and physiological system studied were recorded. For studies that were included in meta-analysis the following data were independently extracted by MG and SFC and verified by JF: name and duration of mission, animal species; animal sample size (n) of spaceflight, ground control, vivarium control, and delayed simulation (when applicable); bone and bone region being measured; and mean, median and median percent difference in the 18 bone health parameters (**Table 1**); standard deviations, standard errors of the mean and/or interquartile ranges; day or range of days when measurements were performed. If the type of measure of the dispersion was not stated, it was assumed to be a standard error, which ensures a conservative estimate. If a range of sample sizes was reported, the smallest value was extracted. Extracted study characteristics for covariate analysis included: animal strain, age, sex, spaceflight group sacrifice delays, single vs grouped spaceflight habitat, space agency, treatment conditions of ground control group, and the presence of sham operations. The information regarding a specific mission was pooled from all applicable articles. When different data for apparently identical samples were presented in two papers, we included the data from the study with the higher quality score. For spaceflight group sacrifice delay, if a range of time was given, the largest time interval was used. Alternate terms used for included parameters are presented in **Table S2**.

Table 1. Bone parameters included in Meta-analysis.

Parameter (abbreviation)	Description	Units
Trabecular (Tb) Bone Measures		
1. Tb. bone volume fraction (Tb.BV/TV)	Fraction of the cancellous space occupied by Tb bone	%
2. Tb. Thickness (Tb.Th)	Mean thickness of trabeculae	mm or µm
3. Tb. Number (Tb.N)	Mean number of trabeculae per unit length	mm^{-1}
4. Tb. Separation (Tb.Sp)	Mean distance between trabeculae	mm or µm
5. Connectivity Density	Number of connected trabeculae per unit volume	mm^{-3}
6. Total bone volume fraction (Total BV/TV)	Total bone volume/ tissue volume	%
Cortical (Ct.) Bone Measures		
1. Marrow Area (Ma.Ar)	Cross-sectional area occupied by medullary canal	mm^2
2. Marrow Diameter (Ma.Dm)	Mean diameter of medullary canal	mm
3. Ct. Thickness (Ct.Th)	Cross-sectional thickness of cortical bone	mm or µm
4. Ct. Bone Area (Ct.Ar)	Cross-sectional area occupied by cortical bone	mm^2
Bone Turnover Measures		
1. Osteoblast Surface (Ob.S/BS)	Percent of bone surface covered with osteoblasts	%
2. Osteoblast Number (N.Ob)	Number of osteoblasts per length of bone {39,43} or per visual field {41}	#/mm #/field
3. Osteoid Surface (OS/BS)	Percentage of bone surface covered in osteoid	%
4. Osteoid Thickness (O.Th)	Mean thickness of osteoid seams	µm
5. Osteoclast Surface (Oc.S/BS)	Percent of bone surface covered with osteoclasts	%
6. Osteoclast Number (N.Oc)	Number of osteoclasts per length of bone {43,48,52} or per visual field {41}	#/mm #/field
7. Bone Formation Rate (BFR)	Volume {36,38,57} or area {43,50,54,56} of bone formed per day, normalized to bone volume {57} or bone length {56}	mm^3/day mm^2/day %/day mm^2/mm/day
8. Mineral Apposition Rate (MAR)	Thickness of new bone formed per day	µm/day

6.3 Measurement-level outcomes

Three types of control group were used: the vivarium control (VC), where animals lived in a standard laboratory habitat; the ground control (GC), where some or all aspects of space flight excluding microgravity were modelled; the delayed simulation (DS), only seen in primate studies, where spaceflight animals were placed in an earth-based GC habitat several weeks following recovery. When available, we used GC as the comparison group. If multiple GC groups were used,

we treat the group that most closely matched flight conditions as the GC. When GC was not available, we used VC or DS as the comparison group. For each individual measurement *j*, we extracted the mean space flight (SF) values, μ_{SF_j}, and the mean comparison control (CC) values, μ_{CC_j} with the corresponding standard errors se_j, or standard deviations sd_j. If sd_j was extracted, it was converted to se_j by dividing by the square root of sample size n of the corresponding group, such as n_{SF} for spaceflight and n_{CC} for comparison control. When median *P* and interquartile range x_{upper}- x_{lower} were given, μ_j was calculated as $\mu_j = (x_{upper} + P + x_{lower})/3$ with: $se_j = x_{upper} - x_{lower}/\sqrt{n} \times 2.7$. For each measurement, we calculated percentage difference, θ_j, between μ_{SF_j} and μ_{CC_j} as: $\theta_j = \frac{\mu_{SF_j} - \mu_{CC_j}}{\mu_{CC_j}} \times 100\%$

Normalized standard errors SE_j were calculated as $SE_j = se_j/\mu_{CC_j}$. The standard deviation for percentage difference of a single measurement σ_j was calculated assuming that the SF and CC groups were independent: $\sigma_j = \sqrt{{SE_{SF_j}}^2 + {SE_{CC_j}}^2} \times 100\%$

6.4 Mission-level outcomes

Data for multiple *b* bones or bone regions presented in one or more studies for the same group of animals were pooled as unweighted averages $\theta_i = \frac{\sum \theta_j}{b}$ to represent the outcome or effect size of a single mission i. In two instances (Bion M1 and SpaceLab 3) where the data for two animal groups on the same mission were reported separately, they were treated as two independent missions. The overall standard error for each mission was calculated as: $SE(\theta_i) = \sqrt{\frac{\sum {\sigma_j}^2}{\sum (n_{SF} + n_{CC})}}$.

6.5 Meta-analytic model and global outcome

Since the mission-level data encompass outcomes from many spaceflights performed over a long period of time in multiple animal species, we rejected the fixed-effect model in favor of random

effects model. However, since individual sample sizes were small (between 4 and 12), the variance is not a representative measure of better estimate of the mean, making variance-based weighting scheme biased. Therefore, to calculate global effect size $\hat{\theta}$, the mission-level outcomes θ_i were weighted by the sample size of spaceflight animals n_{SF}: $\hat{\theta} = \frac{\sum_i \theta_i \times n_{SF}}{\sum_i n_{SF}}$. When combining data from multiple articles with differing sample size n_{SF}, the smallest sample size among them was used for global outcome calculations. Global outcomes were calculated for mice, rats, primates and rodents overall.

To account for heterogeneity between the studies, we adapted the approach developed by Standley and Doucouliagos [32], in which we adjusted the pooled standard error by the factor representing the degree of heterogeneity within the dataset. We calculated the adjusted heterogeneity estimator H^2 to represent the variability of θ_i from the global outcome $\hat{\theta}$ within N mission-level outcomes as follows: $H^2 = \frac{\sum_i (\frac{\theta_i}{SE(\theta_i)} - \frac{\hat{\theta}}{SE(\theta_i)})^2}{(N-1)}$. The standard error of the global outcome $\hat{\theta}$ was then calculated as: $SE(\hat{\theta}) = \sqrt{\frac{H^2}{N}} \times \sqrt[2]{\frac{\sum_i (SE(\theta_i)^2 \cdot (n_{SF}-1))}{\sum_i (n_{SF}-1)}}$.

This meta-analytic model provides the unbiased estimate of the central tendency and conservative estimates for the 95% confidence intervals (CI) which was determined as 95% CI= $\hat{\theta} \pm z_{(1-\alpha/2)} \times SE(\hat{\theta}) = \hat{\theta} \pm 1.96 \times SE(\hat{\theta})$. To assess the influence of spaceflight associated conditions other than microgravity, we similarly calculated percentage difference of GC from VC.

6.6 Rate of change

To estimate the rate of change per day, we used mission-level outcomes from the parameter with the largest dataset, trabecular BV/TV. For each mission, percentage difference in trabecular BV/TV was divided by the duration of each mission $Days$ to calculate $\theta_{i_{per\ day}} = \frac{\theta_i}{Days}$ and

$\mathrm{se}(\theta_i)_{per\ day} = \frac{\mathrm{se}(\theta_i)}{Days}$, which were than used in the meta-analytic model. Although studies in humans have suggested that change in bone mass in space does not occur linearly[5], with only 2 measurements for each group, any rate estimate other than linear would inevitably result in over-fitting.

6.7 Heterogeneity and publication bias analysis

To quantify heterogeneity, we calculated H^2 as described above and I^2 as $I^2 = \frac{H^2-1}{H^2}$. To examine the contribution of individual datasets we used single data exclusion analysis, when one mission-level outcome was excluded and its effect on heterogeneity on the remaining dataset was calculated; and cumulative data exclusion analysis, when multiple mission-level outcomes were excluded in the order of their contributing heterogeneity. To assess publication bias, a funnel plot was used to plot the distribution of the standard errors relative to estimated mission-level outcomes. All the studies were included in the final analysis independent of their contribution to heterogeneity or potential bias.

6.8 Additional analysis

We performed subgroup analysis on 11 characteristics: age of animals, strain of rats, sex of mice, flight duration, individual vs grouped housing conditions, space agency, the conditions of ground control, delay time of SF animal sacrifice, presence of sham operation, quality score of papers and skeletal region of measurements. For strain, sex, space agency, ground control, and housing condition, the subgroup analysis was performed by a categorical value for each mission using the mission-level effect size and 95% CI as described above. For continuous values of age of animals, duration of flights, sacrifice delay and quality score, the missions were divided into 2 groups of approximately equal size for sub-group analysis; or a linear regression against the continuous variable was performed for representative parameters for trabecular and cortical structure and

turnover. For quality score, measurement-level outcomes from a single article were combined to create a paper-level outcome, θ_p and associated measure of variance $SE(\theta_P)$, replacing mission-level outcomes in subgroup analysis and linear regression. For skeletal region, measurement-level outcomes were combined. For quality score and bone region analysis, the global effect size $\hat{\theta}$ and standard error $SE(\hat{\theta})$, were estimated using the random-effects model with the Hedges estimator τ for unit weight $w_i = \frac{1}{SE(\theta_i)^2+\tau^2}$: $\hat{\theta} = \frac{\sum_i(\theta_i \cdot w_i)}{\sum_i(w_i)}$, $SE(\hat{\theta}) = \frac{1}{\sqrt[2]{\sum_i(w_i)}}$ {33}. Subgroup analysis was only performed on parameters with 6 or more mission-level, paper-level or measurement-level outcomes.

6.9 Outcome reporting

Data are presented as effect size or percentage difference between spaceflight and ground control animals or ground control and vivarium control with lower and upper limits of 95% CI as: *ES(%) [lower CI, Upper CI].*

6.10 Software

Endnote X7 and Rayyan were used for the management of references. WebPlot digitizer was used in data extraction. Numbers (version 4.1.1) was used for data management. R (version 1.1.463) was used for meta-analysis and associated calculations. R (version 1.1.463), JASP (version 0.10), and MATLAB (MATLAB online) were used for initial figure preparation.

7. Results

7.1 Overview of relevant studies

The systematic search describing the overlap of space travel, animals, and bone executed in Medline, Embase, Web of Science, and BIOSIS, together with the 9 reports found via manual searches of the NASA Technical Report Server and the compendium of animal and cell spaceflight experiments compiled by Ronca et al. [13] resulted in identification of 1,128 candidate articles (**Fig. 1A**). Of these, 340 articles focused on bone, while the rest discussed a range of physiological systems potentially relevant to bone heath, including skeletal muscles, metabolism and developmental issues (**Fig. 1B**). The majority of studies (83%) described findings in rats (664/1,128), mice (181/1,128) and primates (96/1,128) (**Fig. 1C**). The number of papers describing animals in space peaked in the 1990s (**Fig. 1D**). From the 1970s through the 2000s, rats were the main spacefaring animal model. Interest in primates peaked in the 2000s, however, in the last decade mice have become the predominant animal model studied in space (**Fig. 1E**). Considering the available data, the full text screen focused on 340 studies describing bone health in rodents and primates, and identified 63 studies that presented quantitative measurements of trabecular and cortical bone architecture or bone turnover[34-96]. After excluding studies that reported data on treated animals, reported duplicate data or demonstrated unclear reporting (**Table S3**), 40 articles were selected for the final meta-analysis: 23 describing rats[34-56], 12 describing mice [57-68], 4 describing primates [69-72], and 1 describing both mice and primates [73]. The final dataset included a total of 95 rats, 61 mice and 9 primates (rhesus macaque monkeys) flown to space on 22 missions (**Table 2**).

Table 2. Description of articles included in the meta-analysis.

Year	Mission	Days	Article	Species	n_{SF}	Type of Control	Bones Analyzed (sub-sections)	QS (/25)
1975	Cosmos 782	19.5	Asling 1978	Rats	6	GC, VC	Tibia (M)	**13***
			Morey 1978		11	GC, VC	Tibia (D)	**20**
1977	Cosmos 936	18.5	Morey-Holton 1978	Rats	10	GC, VC	Tibia (D)	**18***
1979	Cosmos 1129	18.5	Judy 1981	Rats	7	VC	Tibia (M)	**14***
			Wronski 1981		11	GC, VC	Rib(NS), Humerus (D), Tibia (D)	**20***
			Jee 1983		7		Humerus (M), Tibia (M)	**19**
			Rogacheva 1984		6		Femur (D)	**12.5**
1983	Cosmos 1514	5	Cann 1986	Primate	1	GC	Ulna (D), Radius (D), Tibia (D)	**14.5***
1985	Cosmos 1667	7	Kaplanskii 1987	Rats	7	GC, VC	*Vertebrae (L)*, Pelvis (Ilium), Tibia (D, *M*)	**15.5**
			Vico 1988			GC	Vertebrae (T8, L1), Femur (M), Tibia (M)	**17**
1985	SpaceLab3	7	Wronski 1987	Rats	L5 S6	GC	Vertebra (L4), Humerus (M), Tibia (D)	**19.5**
1987	Cosmos 1887	12.5	Vailas 1990	Rats	5	GC, VC	Humerus (D)	**20**
			Doty 1990				Tibia (D)	**19**
			Zerath 1990			VC	Vertebrae (NS), Humerus (M)	**14**
			Cann 1990	Primates	2	DS, VC	Ulna (D), Radius (D), Tibia (D)	**16.5***
1989	Cosmos 2044	14	Zerath 1991	Primates	2	DS	Pelvis (Ilium)	**15.5**
				Rats	5	GC, VC	Vertebra (T9), Humerus (M)	**18**
			Vailas 1992				Humerus (D)	**19**
			Vico 1993				Vertebra (L2, T5), Femur (M), Tibia (E,M)	**20**
1992	Bion 10	11.5	Zerath 1996b[{71}]	Primates	2	DS, GC, VC	Pelvis (Ilium)	**20**
1992	STS-52	10	Turner 1995	Rats	6	GC	Humerus (M)	**16**
1992	STS-57	11	Westerlind 1995	Rats	12	GC, VC	Femur (D), Tibia (M)	**23**
1993	STS-58 (SLS-2)	14	Zerath 1996a[{51}]	Rats	5	GC, VC	Vertebrae (T9,C7), Humerus (M)	**23**
			Lafage-Proust 1998				Femur (M), *Humerus (M)*	**18**
1996	Bion 11	14	Zerath 2002	Primates	2	GC, VC	Pelvis (Ilium)	**21**
1996	STS-77	10	Bateman 1998	Rats	6	VC	Humerus (D), Tibia (D)	**20**
1996	STS-78	17	Wronski 1998	Rats	6	GC, VC	Vertebra (L1), Tibia (M, D)	**24**
			Zerath 2000a[{55}]				Vertebra (T8), Pelvis (Cotyloid)	**22**
			Vajda 2001				Femur (D)	**21**
2001	STS-108	12	Lloyd 2015	Mice	12	GC	Vertebra (L5), Humerus (M), Femur (D), Tibia (M)	**21**
2007	STS-118	13	Ortega 2013	Mice	12	GC	Femur (M), Tibia (M, D)	**18.5**

2010	STS-131	15	Blaber 2013	Mice	7	GC	Pelvis (Ischium), Femur (M)	**19**
			Zhang 2013				Calvaria	**16.5**
			Blaber 2014		8		Femur (E, M)	**17.5**
2013	Bion M1	30	Berg-Johansen 2016	Mice	3	VC	Vertebrae (C)	**16**
			Macaulay 2017		6	GC	Calvaria	**21**
			Gerbaix 2017		5		Vertebrae (L1,L3,T12), Femur (M,D)	**20**
			Gerbaix 2018				Calcaneus, Navicular, Talus	**17**
2016	SpaceX CRS-9	39	Shiba 2017	Mice	5	GC	Femur (prox)	**17.5**
2017	SpaceX CRS-10	28	Maupin 2019	Mice	10	GC	Calvaria, Rib (10), Sternum, Vertebra (L4), Humerus (M,D), Femur (M,D), Tibia (M,D)	**21**
2017	SpaceX CRS-12	34	Tominari 2019	Mice	3	GC	Humerus (prox), Tibia (prox)	**17**

Days = mission duration (days); n_{SF}= sample size of spaceflight animal group. Control groups: GC = ground control, VC = vivarium control, DS = delayed stimulation. Sub-sections of bones analyzed: E = epiphysis, M = metaphysis, D= diaphysis, prox = proximal. For vertebrae region: L = lumber, T = thoracic, C = caudal, NS = not specified. *Italics* indicates overlapping bones measured excluded from the meta-analysis. QS = quality score calculated according to Supplemental Information S2. *Indicates articles sourced from NASA Final Reports of Soviet missions. For the specific measurements present in each study, refer to **Table S4**. For rodent study characteristics used for covariate analysis, refer to **Table S5**

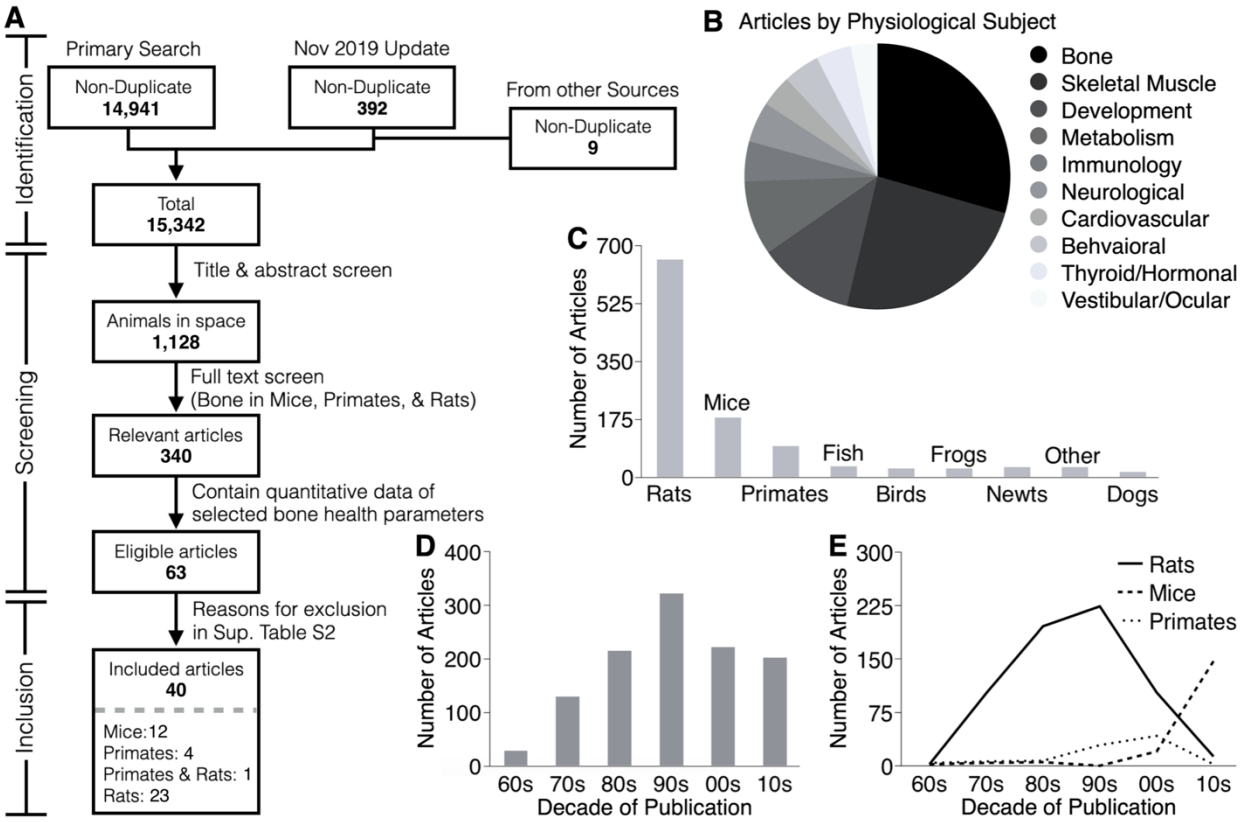


Figure 1. Systematic review information flow and outcomes. (A) Prisma diagram. B-E) Analysis of 1,128 articles selected after title and abstract screening. (B) Distribution of physiological systems mentioned in the papers. (C) Number of articles discussing indicated species. (D) Number of articles by publication decade. (E) Number of articles by publication decade for species of rats (solid line), mice (dashed line) and primates (dotted line).

7.2 Heterogeneity, bias and the meta-analytic model

Statistical heterogeneity was moderate to high (I^2 > 46%) for all the extracted parameters for spaceflight-related changes except for bone marrow area (I^2 = 14.4%) and cortical bone area (I^2 = 0%). Single mission exclusion analysis identified some mission-level outcomes removing which reduced the overall heterogeneity, however no single mission influenced the heterogeneity of more than one parameter, or the global outcome for Tb.BV/TV or Tb.N parameter datasets (**Fig. 2A**, Supplemental **Fig. S1A**). Cumulative-mission exclusion analysis demonstrated that exclusion of >21% of missions leads to a homogeneous ($I^2 \leq 30\%$) dataset, however the overall outcomes for

Tb.BV/TV and Tb.N were not affected by decreased heterogeneity (**Fig. 2B**, **Fig. S1B**). The funnel plot demonstrated symmetrical distribution (**Fig. 2C, Fig. S1C**). We assessed the quality of individual papers on a 25-point scale (Supplemental Information S2) and examined if quality score affected the reported paper-level variance (**Fig. 2D, Fig. S1D**) or effect size (**Fig. 2E**), however, no significant association of quality score with reported outcomes was observed. Subgroup analysis further demonstrated no difference between papers with low (< 20) and high (≥ 20) quality score (**Fig. S2**). We conclude that the publication bias is negligible within this dataset. To account for low sample sizes as well as heterogeneity, we used the modified sampling by size method [97] for further analysis.

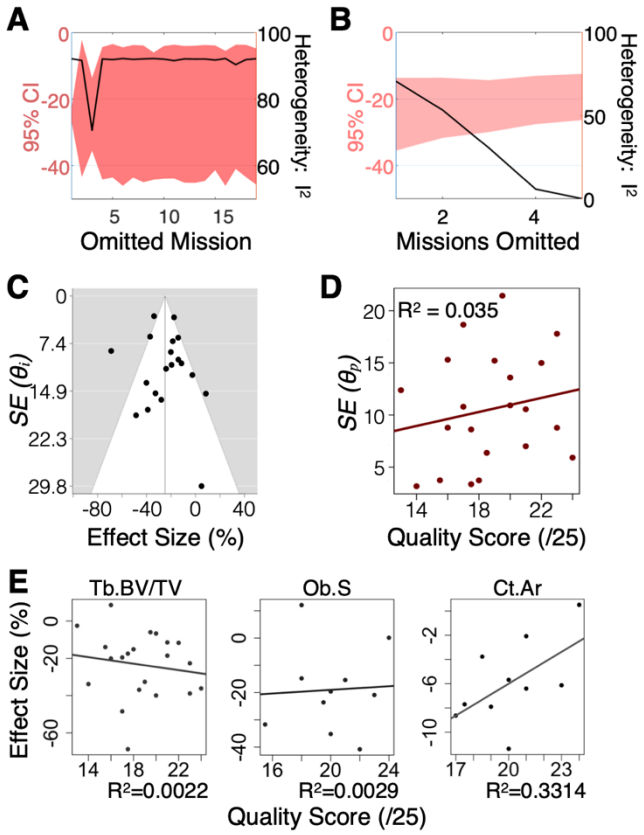


Figure 2. Heterogeneity and sensitivity analyses for the BV/TV dataset. A, B) Heterogeneity was analyzed using single mission exclusion (A) and cumulative mission exclusion (B). Red area: 95% CI for the global effect size (left axis); line: I^2 (right axis). C) Funnel plot; D) article-level standard error $SE\ (\theta_P)$ as a function of quality score. E) Meta-regression of the Tb.BV/TV, Ob.S, and Ct.Ar paper-level outcomes as a function of quality score. Maximum quality score was 25. R^2 is shown.

7.3 Changes in trabecular architecture during spaceflight

Many studies included two types of control –vivarium control (VC), where animals lived in a standard laboratory habitat, and the ground control (GC), where some or all aspects of space flight other than microgravity, such as physical enclosure, diet and lift off and re-entry forces, were simulated. We examined the percentage difference in spaceflight compared to GC, as well as in GC compared to VC. Of the 6 parameters describing trabecular bone: trabecular bone volume fraction (Tb.BV/TV), thickness (Tb.Th), number (Tb.N), separation (Tb.Sp), connective density and Total BV/TV; Tb.BV/TV was significantly lower in spaceflight mice and rats compared to ground control, and Tb.Th was significantly reduced for mice (**Fig. 3A,B left**). For rodents overall, Tb.BV/TV and Tb.Th changed significantly by -24.1% [-43.4,-4.9] and -9.0% [-12.9,-5.2] respectively. Tb.N, Tb.Sp, and connective density demonstrated trends towards poor bone health in spaceflight mice and rats, however only the change in Tb.N reached statistical significance (**Fig. 4A-C**). Total BV/TV, which was measured in flat bones and in one case vertebra, did not change due to spaceflight (**Fig. 4D**). When ground and vivarium controls were compared, Tb.BV/TV, Tb.N, and Tb.Sp were unaffected, but Tb.Th was significantly lower in GC compared to VC (**Fig. 3A,B right**, **Tables S6, S7**), suggesting that flight conditions other than microgravity may contribute to a reduced Tb.Th. In all trabecular parameters in rodents, heterogeneity was moderate to high, I^2>46%. Trabecular parameters were measured in 4 primates on missions Bion 10 and 11, and demonstrated significantly lower Tb.BV/TV, a trend to reduced Tb.N, and Tb.Th, and a trend to higher Tb.Sp compared to GC (**Table 3**). Thus, there was a deficit in trabecular bone in rodents and primates after the spaceflight.

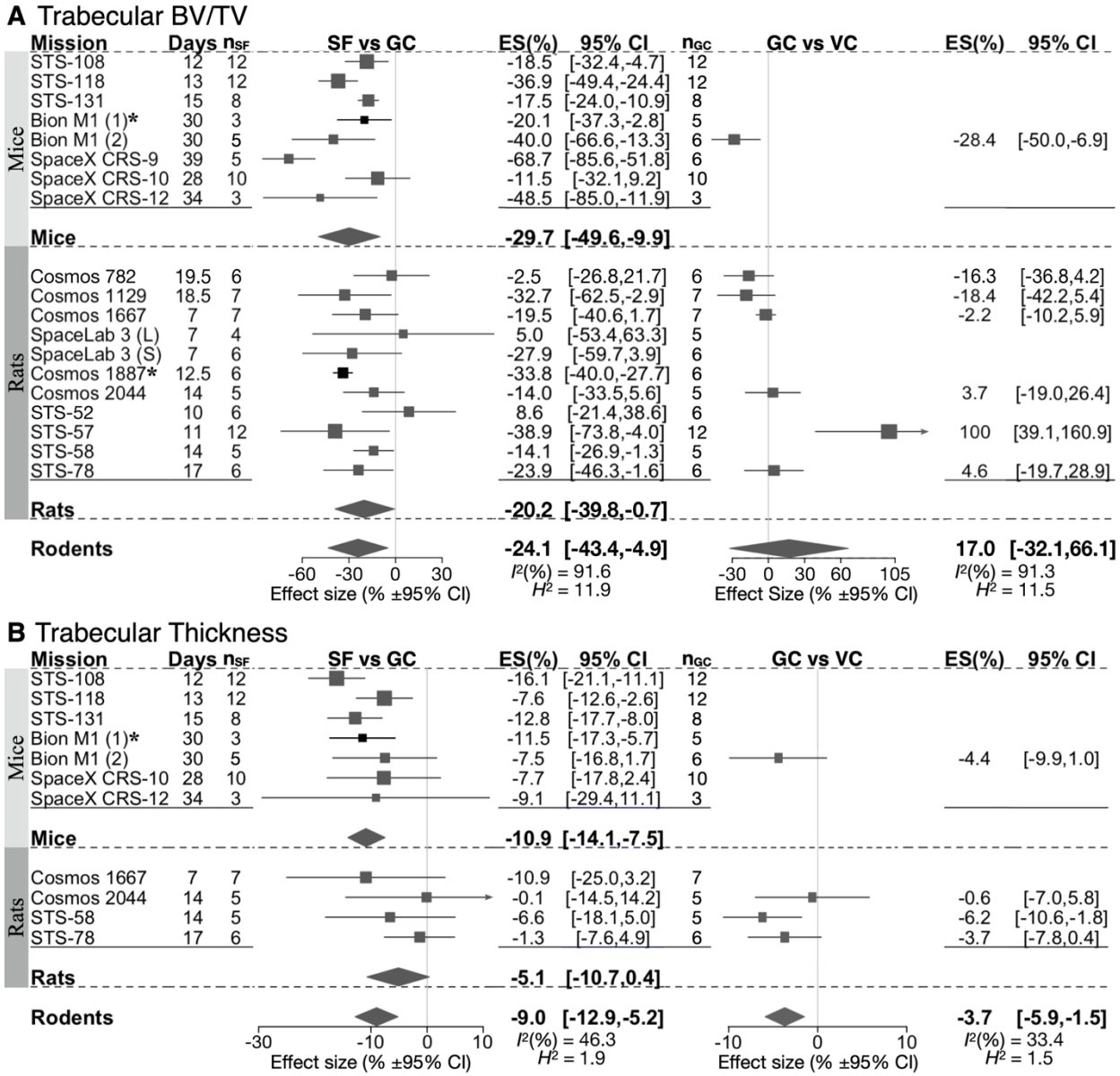


Figure 3. Forest plot of spaceflight and ground control-induced changes to Tb.BV/TV and trabecular thickness. Changes in BV/TV (A) and trabecular thickness (B) of spaceflight animals (SF) compared to ground control animals (GC) (Left); and GC compared to vivarium controls animals (VC) (Right). For each indicated species, missions are sorted by mission year (old to new); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI, the size of the square is proportional to n_{SF}. Overall effect size (%) and 95% CI are indicated by diamonds for mice, rats and rodents, I^2, and H^2 are given for rodents. * indicate missions wherein GC was not present, and SF was compared to VC.

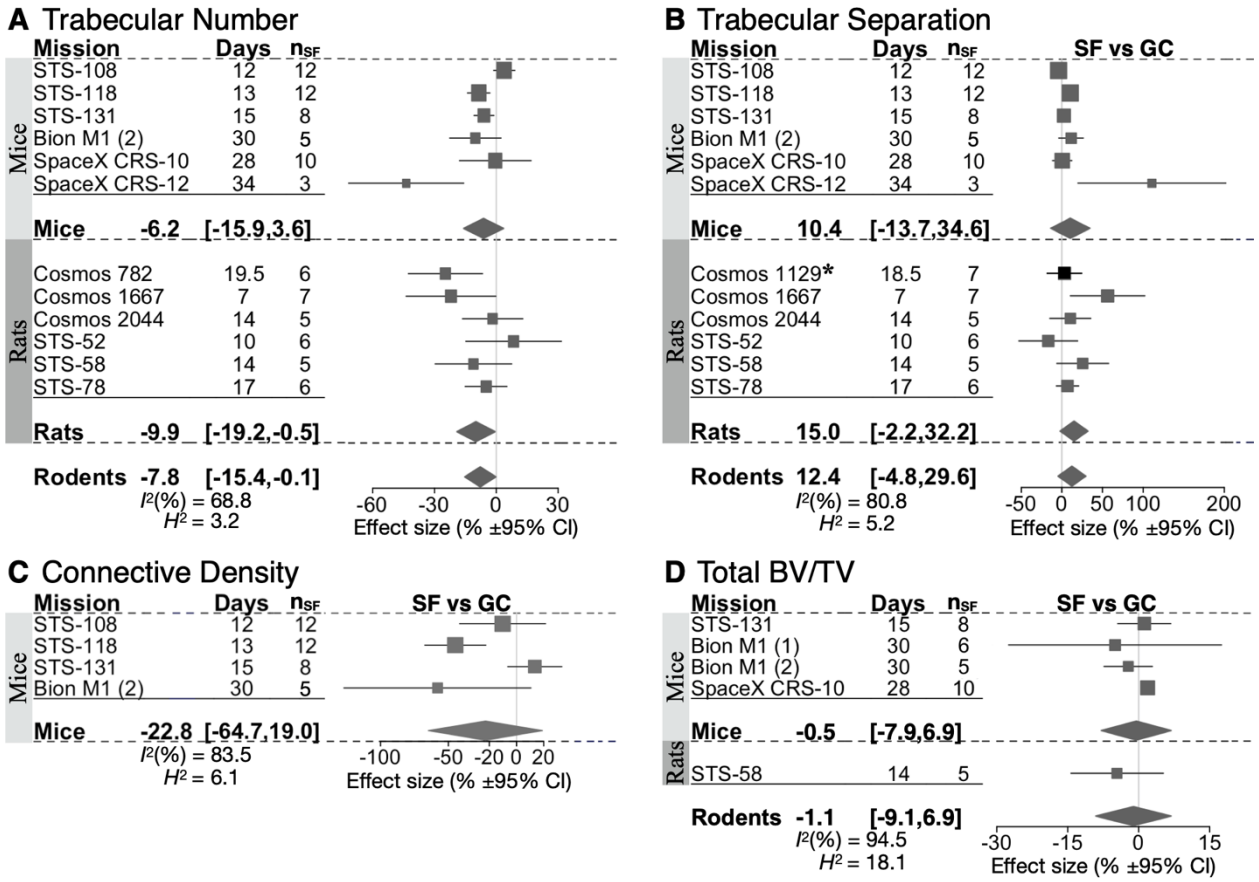


Figure 4. Forest plot of spaceflight induced changes to trabecular number, trabecular separation, connective density and total BV/TV. Changes in trabecular number (A), trabecular separation (B), connective density (C) and total BV/TV (D) of space flight animals (SF) compared to ground control animals (GC). For each indicated species, missions are sorted by mission year (old to new); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI, the size of the square is proportional to n_{SF}. Overall effect size (%) and 95% CI are indicated by diamonds for mice, rats and rodents, I^2, and H^2 are given for rodents. * indicate missions wherein GC was not present, and SF was compared to VC. For mission-level effect sizes and 95% CI, refer to Supplemental Tables S6-9.

7.4 Changes in trabecular bone turnover during spaceflight

We next examined if spaceflight-induced bone deficits are associated with abnormal function of osteoblasts or osteoclasts. Osteoid surface (OS) and thickness (O.Th) were significantly lower in rodents by -29.9% [-53.9,-5.8] in OS and -28.6 [-54.5,-2.7] in O.Th; Osteoblast surface (Ob.S) and osteoblast number (N.Ob) demonstrated a trend to decrease compared to GC (**Fig. 5**). Comparison of ground and vivarium controls was available for only two missions, except for Ob.S, which was

not significantly different (**Tables S10-S13**). Heterogeneity for osteoblast parameters was moderate to high I^2>50%. The osteoblast parameters were from trabecular skeletal regions, except for missions Cosmos 936 (Ob.S) and Cosmos 1667 (OS and O.Th), in which the measurements were from endocortical surface of the tibia diaphysis and metaphysis respectively, and excluding these data resulted in a homogeneous datasets for Ob.S and O.Th (I^2 = 0), but not for OS (I^2 = 74.9%). When only osteoblast indices in trabecular bone are considered, spaceflight resulted in a statistically significant reduction in Ob.S of -20.1% [-35.0, -5.1], OS -30.4% [-55.1, -5.8] and O.Th -36.2% [-60.2,-12.2]. Thus, osteoblast formation and function in rodents were negatively affected by spaceflight.

In contrast to osteoblast parameters, changes in osteoclasts were inconsistent. Osteoclast surface (Oc.S) in SF mice demonstrated large study level increases in 2 of 3 datasets, however it was unaffected in SF rats (**Fig. 6A left**). Osteoclast number (N.Oc) was higher in the one group of SF mice where it was measured, and was not significantly affected in spaceflight rats (**Fig. 6B left**). Moreover, comparing ground and vivarium controls demonstrated strong (10-70%) tendencies for study level increases (**Fig. 6 right**). Although the overall effect size for GC vs VC comparisons only reached statistical significance for Oc.N, these data suggest that in rodents osteoclasts may be affected by spaceflight conditions other than microgravity. Heterogeneity was high for Oc.S and N.Oc datasets. The osteoclast parameters were from trabecular skeletal regions, except for missions Cosmos 936 (N.Oc) and one of the bones for mission Bion M1 (Oc.S); excluding these data did not significantly change the outcome. Thus, osteoclast parameters were unaffected in rats and variably affected in mice.

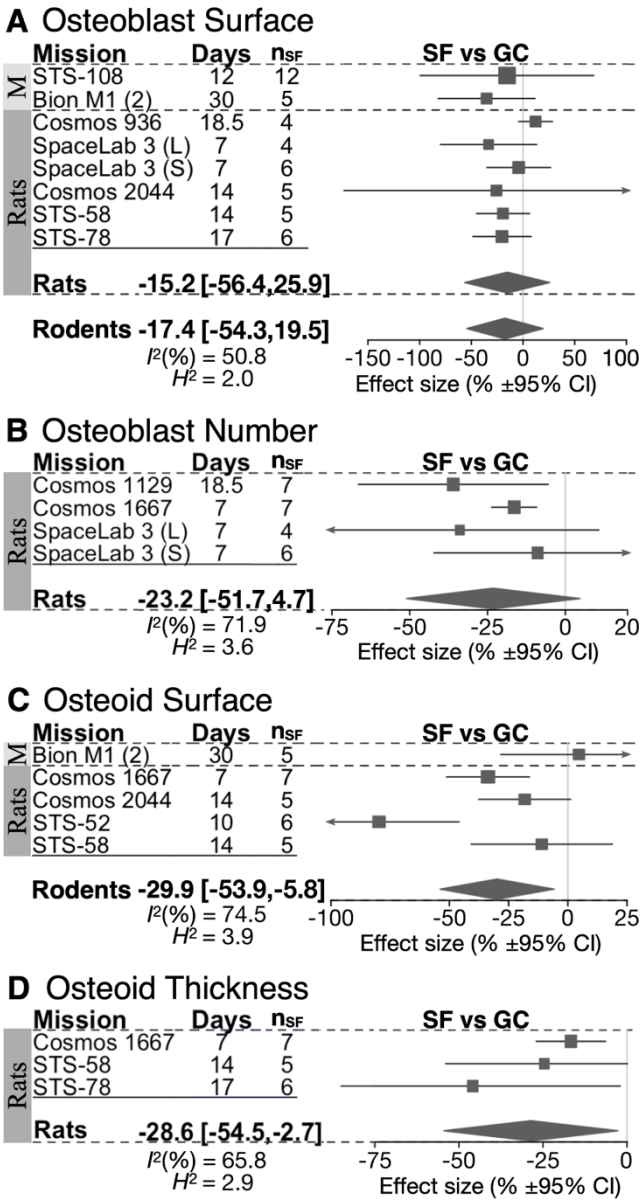


Figure 5. Forest plot of spaceflight induced changes in trabecular bone turnover parameters. Changes in osteoblast surface (A), osteoblast number (B), osteoid surface (C), and osteoid thickness (D) of space flight animals (SF) compared to ground control animals (GC). For each indicated species, missions are sorted by mission year (old to new); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI, the size of the square is proportional to n_{SF}. Overall effect size (%) and 95% CI are indicated by diamonds for rats and rodents, I^2, and H^2 are given for rodents.

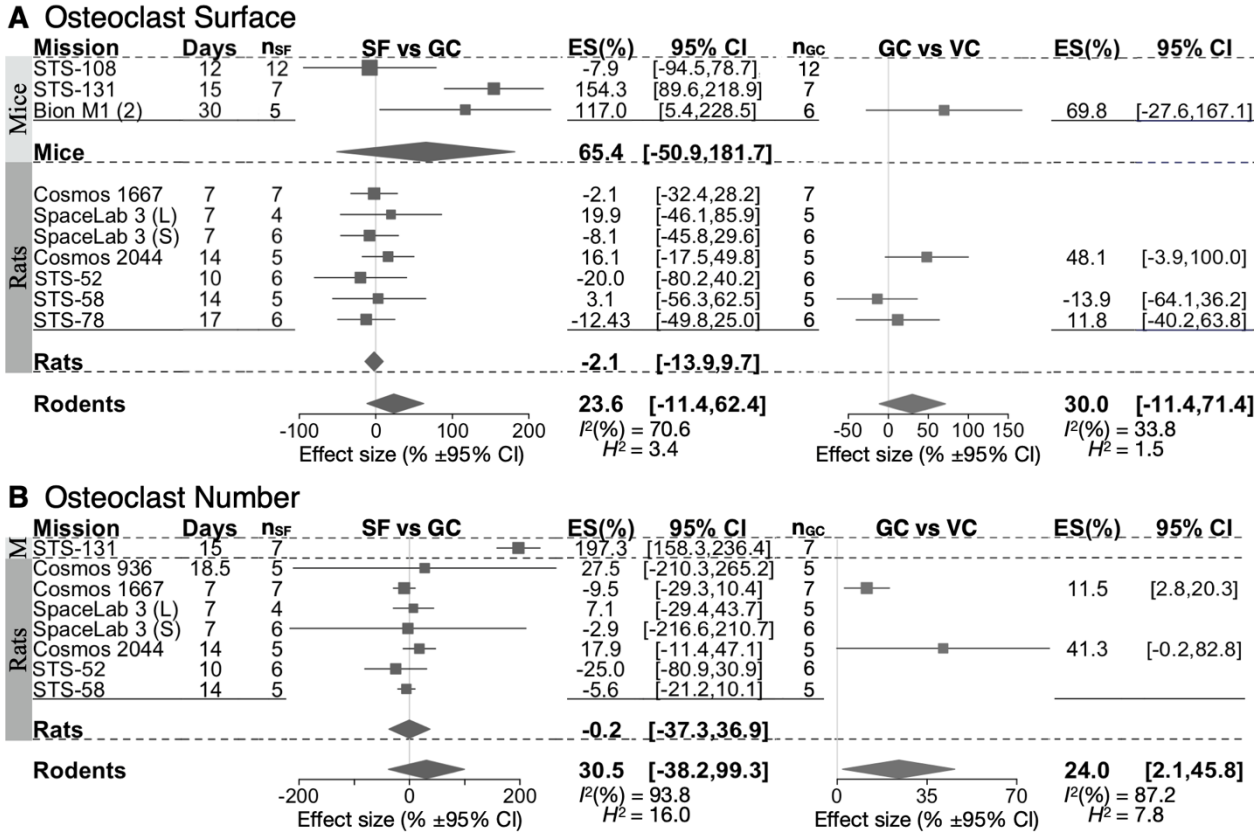


Figure 6. Forest plot of spaceflight induced changes to osteoclast parameters. Changes in osteoclast area (A), and osteoclast number (B) of space flight animals (SF) compared to ground control animals (GC) (Left); and GC compared to vivarium controls animals (VC) (Right). For each indicated species, missions are sorted by mission year (old to new); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI, the size of the square is proportional to n_{SF}. Overall effect size (%) and 95% CI are indicated by diamonds for mice, rats and rodents, I^2, and H^2 are given for rodents.

7.5 Change in cortical bone architecture during spaceflight

Cortical parameters analyzed were bone marrow area (Ma.Ar, which included data on bone marrow diameter (Ma.Dm) transformed as $\pi(d/2)^2$), cortical area (Ct.Ar) and thickness (Ct.Th). Ma.Ar and Ct.Th did not significantly differ between SF and GC in mice and rats, while Ct.Ar was significantly lower in spaceflight mice and rats (**Fig. 7A-C**). GC did not significantly differ from VC for any cortical parameter (**Tables S14-S16**). The heterogeneity for cortical parameters was low, I^2<15%, except for Ct.Th which showed high heterogeneity, I^2=90.7%. The datasets of

Ma.Ar, Ct.Ar, and Ct.Th are composed of measures taken in the diaphysis of long bones except for missions STS-131 (femoral neck), Bion M1 (animal group 1, ankle bones and calcaneus) and SpaceX CRS-10 (rib). Removing these biological outliers did not affect effect size and resulted in a homogeneous dataset for Ma.Ar with I^2=0%. Cortical thickness measured in 4 primates was not significantly affected by spaceflight (**Table 3**). Thus, spaceflight resulted in cortical bone deficits, however it was affected to a smaller degree compared to trabecular bone.

7.6 Change in cortical bone turnover during spaceflight

Only measures of bone formation rate (BFR) and mineral apposition rate (MAR) from the cortical bone surface in the diaphysis of long bones were included in analysis. This resulted in the exclusion of measures of MAR and BFR in the pelvis and thoracic vertebrae from STS-78[{55}], and in the humeral metaphysis from STS-52 and non-included mission STS-41[{74}]. Both BFR and MAR were lower in spaceflight rodents by -34.2% [-50.2,-12.8] and -13.5% [-27.1,0.1] respectively (**Fig. 7D,E**). There were no differences between GC and VC for BFR nor MAR (**Tables S17, S18**). Heterogeneity was moderate to high for these parameters, I^2 >52%. When long bone measurements of MAR and BFR taken on the periosteal and endocortical surfaces were separated, we found that the reductions in MAR and BFR were only significant on the periosteal surfaces (**Fig. 7F**). Thus, bone formation on periosteal surfaces of cortical bone appears to be more affected by microgravity.

Table 3. Spaceflight-induced changes in bone parameters of primates.

Missions	$\sum n_{SF}/\sum n_{GC}$	SF vs GC		GC vs VC	
		ES (%)	95% CI	ES (%)	95% CI
Tb.BV/TV					
Bion 10 Bion 11	4 / 7	-25.2	[-35.6,-14.7]	-4.6	[-27.4,18.3]
Tb.Th					
Bion 10 Bion 11	4	-14.7	[-34.1,4.8]	-	-
Tb.N					
Bion 10 Bion 11	4	-7.8	[-16.9,1.4]	-	-
Tb.Sp					
Bion 10 Bion 11	4	8.5	[-12.3, 29.2]	-	-
Ct.Th					
Cosmos 1514 Cosmos 1887* Bion 11	5 / 4	-6.4	[-84.2,71.4]	0.8	[-5.4,7.0]
MAR					
Bion 10 Bion 11 Cosmos 2044*	6 / 10	-31.4	[-62.0,-0.7]	1.8	[-2.7,6.2]

*missions in which ground control (GC) was not present and delayed simulation (DS) was used as GC, which were also excluded from GC vs VC calculations. $\sum n_{SF}/\sum n_{GC}$ = total sample size of all spaceflight (SF) groups / all GC groups. SF vs GC: percentage difference between spaceflight and comparison control. GC vs VC: percentage difference between ground and vivarium control.

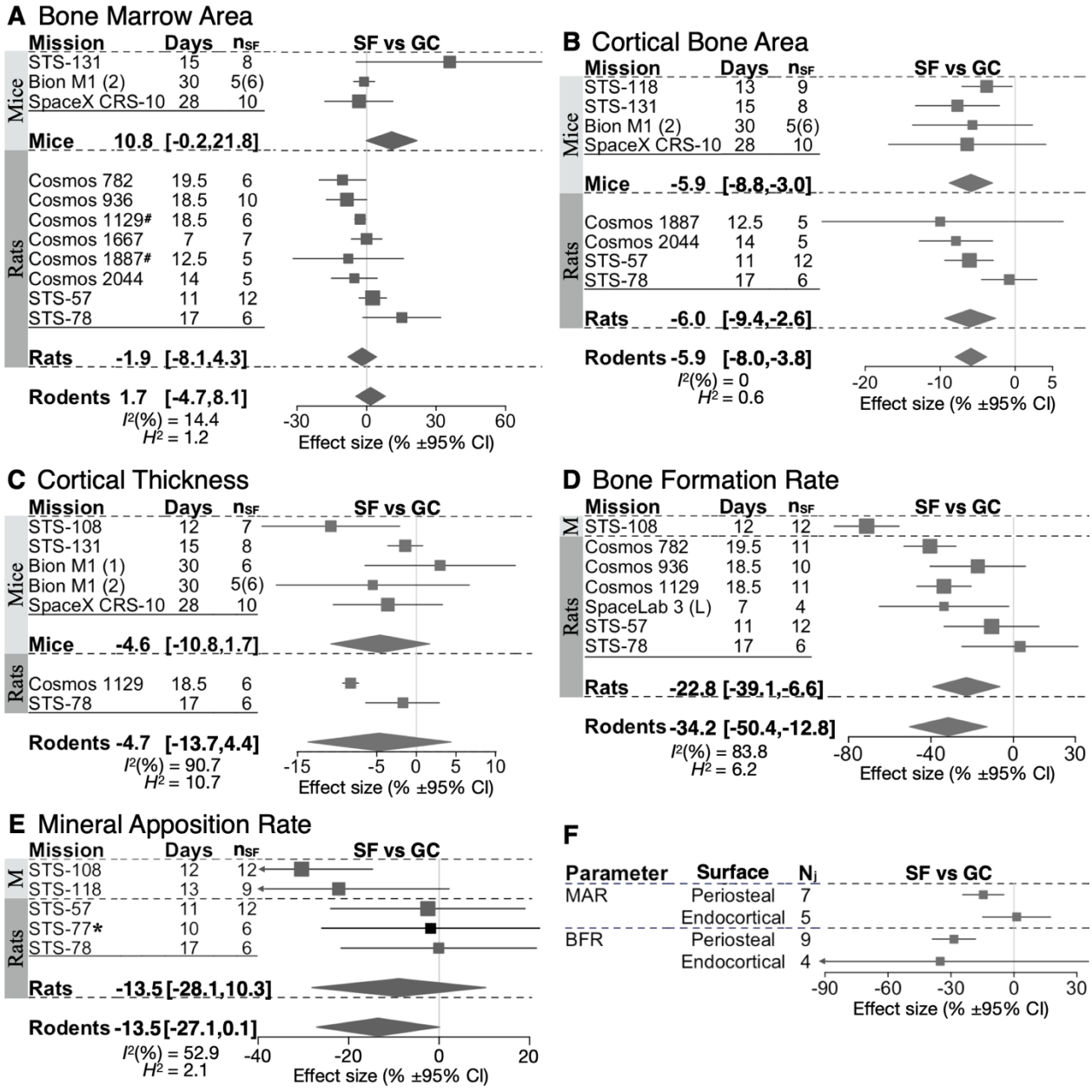


Figure 7. Forest plot of spaceflight induced changes to cortical bone parameters. (A-E) Changes in bone marrow area (A), cortical bone area (B), cortical thickness (C), as well as bone formation rate (D) and mineral apposition rate (E) for the diaphyses of long bones, of space flight animals (SF) compared to ground control animals (GC). For each indicated species, missions are sorted by mission year (old to new); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI, the size of the square is proportional to n_{SF}. Overall effect size (%) and 95% CI are indicated by diamonds for mice, rats and rodents, I^2, and H^2 are given for rodents. * missions where SF was compared to VC. # mission where Ma.Ar was derived from average marrow diameter (Av.Ma.Dm) as $Ma.Ar = \pi(Av.Ma.Dm/2)^2$. F) Change in BFR and MAR on the periosteal and endocortical surface of long bones in SF compared to GC. Number of measurement (N_j) is indicated. Square/line: overall effect size (%) and 95% CI.

7.7 Effects of covariates on spaceflight related changes in animal bone health

We next examined the contribution of covariates to the overall outcomes using sub-group analysis and meta-regression. First, we examine if animal characteristics, such as age, sex and strain affect the overall outcome. Using linear regression analysis, we have found that rodent age was weakly associated with changes in osteoblast surface and cortical area, but not with Tb.BV/TV (**Fig. 8A**). Subgroup analysis further demonstrated that in animals 10 weeks of age or older, larger changes were observed in Tb.N, Ob.S and Oc.S, while Ct.Th was more affected in younger animals (**Fig. S3A**). When we compared trabecular parameters in instances when both primary and secondary spongiosa of a single bone were analyzed, we observed that changes in Tb.BV/TV, Tb.N, and Tb.Th in secondary spongiosa were greater than in primary spongiosa (**Fig.8B**). Animal sex or strain did not significantly affect the outcome (**Fig. S3B,C**).

Next, we examined if mission-related differences affected the outcome. Spaceflight duration did not significantly correlate with changes in Ob.S and Ct.Ar, but was weakly associated with changes in Tb.BV/TV when assessed using meta-regression (**Fig. 8C**). Moreover, subgroup analysis by mission durations shorter or longer than 2 weeks, demonstrated no significant difference between groups for any parameter (**Fig. S4A**). To estimate the rate of accumulation of bone deficits in space, we divided individual outcomes of our largest parameter dataset, Tb.BV/TV, by the mission duration. Although not statistically significant, the deficits in Tb.BV/TV per day were smaller in long spaceflights than in short spaceflights (**Fig. 8D**). We estimated the rate of accumulation of trabecular bone deficits as -1.7%/day [-3.5,0.2], or -1.0%/day [-1.7,-0.4] when taking into account only long duration missions. We also assessed if individual vs group housing affects the outcomes, however no differences were found except for Tb.N, which changed significantly greater when animals were housed individually (**Fig. S4B**). Comparing

outcomes by space agency, we determined no significant difference between space agencies (**Fig. S4C**).

Study-related differences included measurement techniques, presence of sham operation, sacrifice delay and ground control conditions. For all trabecular and cortical architectural parameters, the division of measurement technique (Histology vs μCT) coincided with the species difference of rats and mice preventing us from conducting any further meaningful subgroup analysis. In sham-operated rodents Tb.Th was affected significantly less than in naïve animals (**Fig. S5A**). The sacrifice delay did not significantly affect the outcomes in subgroup analysis, although change in Ob.S was associated with prolonged sacrifice delay in meta-regression analysis (**Fig. S5B, S6**). When ground control groups were divided by the degree to which they mimic the aspects of spaceflight other than the microgravity, we observed no association between the fidelity of the GC and spaceflight-induced changes, suggesting that they were primarily driven by the microgravity (**Fig. S5C**).

In astronauts, bone loss is strongly affected by its position in relation to the gravitational vector [5,98]. To assess if similar trend is present in rodents, we performed sub-group analysis of bones from different regions: region 1 that included calvaria, vertebrae, ribs and sternum; region 2 with pelvis, humerus and femur; and region 3 with tibia and ankle bones (**Fig. 8E left**). Changes in trabecular parameters were larger in bones located more distal from the axial skeleton (**Fig. 8E right**), however the mean effect sizes were not significantly different between the regions. Among other parameters, Ct.Th, Ob.N, OS and BFR demonstrated significant changes only in regions 2 and/or 3, while changes in Ob.S were only significant in regions 1 and 2 (**Fig. S7**). These data suggest that bone position in relation to the gravitational vector may be important for rodents, however targeted studies investigating these relationships would be required.

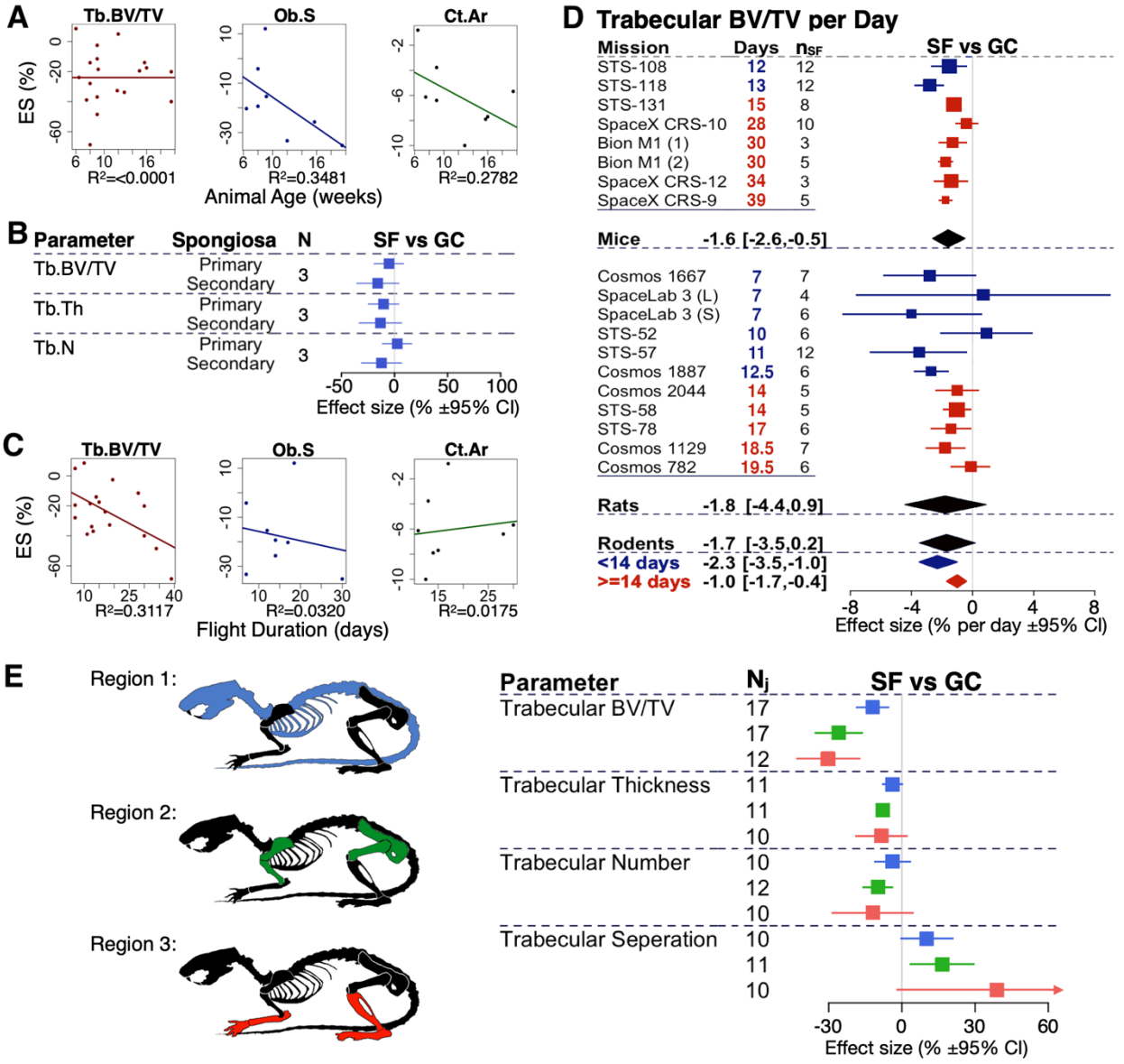


Figure 8. Exploratory analysis for the effects of covariates on spaceflight-induced changes in bone parameters. (A) Meta-regression of the Tb.BV/TV, Ob.S, and Ct.Ar as a function of animal age. (B) Subgroup analysis for Tb.BV/TV, Tb.Th, and Tb.N outcomes for primary and secondary spongiosa. (C) Meta-regression of the Tb.BV/TV, Ob.S, and Ct.Ar as a function of flight duration. (D) Forest plot of the rate of spaceflight induced change to Tb.BV/TV. (E) Subgroup analysis for Tb.BV/TV, Tb.Th, Tb.N, and Tb.Sp outcomes reported for individual rodent bones from region 1 (skull, vertebra, and thorax, blue), region 2 (pelvis, humerus, and femur, green) or region 3 (tibia and ankle bones, red) as illustrated on the left. For A and C, R^2 is shown. For B to E, N = number of missions, n_{SF} = spaceflight animal sample size, and N_j = number of measurements. Square/line: overall effect size (%) and 95% CI. For D, in each indicated species, missions are sorted by duration (shortest to longest); duration of spaceflight (Days) and number of spaceflight animals (n_{SF}) are indicated. Square/line: effect size (%) and 95% CI; dark blue: missions less than 14 days; dark red: missions 14 days or longer. Overall effect size (%) and 95% CI are indicated by diamonds for mice, rats and rodents (black), rodents on short duration (dark blue), and long duration (dark red) missions.

8. Discussion

We systematically reviewed and quantitatively synthesized literature on bone health in space-faring rodents and primates. We report that bone mass is lower in spaceflight rodents and primates, with indications that microgravity is the driving factor inducing bone deficits. Deficits in trabecular bone were larger than in cortical bone and subgroup analysis suggested that distal skeleton was affected more than axial. Osteoblast indices in rodent trabecular bone were significantly lower, however osteoclast numbers were not affected in rats, and were variably affected in mice. Even though the degree of bone deficit was found to poorly correlate with mission duration, the rate of accumulation of trabecular bone deficit was estimated as 1.7% [-3.5,0.2] per day, which is much higher than the estimates of bone loss available for humans. Taken together, our data indicate that microgravity induces bone deficits in rodents and primates, and the data suggest that the prevalent mechanism is suppression of bone formation.

We have found that during the 4-39 days space mission rodents accumulated the deficit of -24.1% [-43.4,-4.9] in trabecular bone tissue, which translates to the rate of 1.7% of trabecular bone deficit per day. In the much smaller dataset for primates, the bone deficit after 11.5-14 day missions was equally high, -25.2% [-35.6,-14.7] or 1.9% per day. These estimates for trabecular bone deficits in spaceflight rodents and primates are much greater than estimates of bone loss for astronauts which have been reported as 0.7-2.7% per month {4,5,99,100}. Nevertheless, similar deficits of 15%-50% in tibial and femoral trabecular bone volume were reported in 2-4 week long hindlimb unloading studies in rats and mice {101-105}, which can be recalculated to 1.1%-3.5% per day. We observed that no single parameter was strongly associated with mission duration. In astronauts, changes to bone were also highly variable for missions less than 30 days in duration {5}. Of spaceflights studying bone in rodents, only 3 missions were longer than 30 days, one of

which (Mice Drawer System (MDS)) was excluded since of the 3 wild type mice aboard, only 1 returned to Earth alive {94}, preventing us from extracting meaningful quantitative data from it. Thus, continuous measurements of bone parameters in longer missions (>30 days) are required to determine the dynamic association between the duration of exposure to microgravity and bone health.

We have identified several instances of notable regional difference in bone response to microgravity. First, we have found that the deficits in trabecular bone were much greater than that in cortical bone in space-traveling rodents. Similarly, higher deficits in trabecular bone compared to cortical were reported in studies of hindlimb unloaded rats {106} as well as in astronauts {99}. In cortical bone, bone formation was only significantly suppressed on the periosteal surface, which is supported in the observation that Ct.Ar, but not Ma.Ar, was significantly lower in spaceflight rodents. Similar changes in cortical bone formation were observed in hindlimb unloading studies in mice {106}. Within trabecular bone, we found that rodents exhibited the relatively greater deficits in secondary spongiosa compared to primary spongiosa. Secondary spongiosa was also found to be more affected compared to primary in rats after hindlimb unloading {85,107,108}. However, in the model of immobilization due to sciatic denervation, the bone loss was isolated to primary spongiosa {109}. Of interest, we also observed a weak association of osteoblast suppression and cortical bone loss with older age in space-traveling rodents. In contrast, extensive and well-controlled studies of the impact of age on bone health in hindlimb unloaded rats reported the opposite trend – higher bone deficits in younger animals {101,104}. In this regard, it is important to note that the oldest spaceflight rodents were relatively young, 20 weeks of age at the start of the mission, and therefore more studies are needed to fully understand the impact of age on bone health in space. Similarly, even though dramatic sex-related differences were reported in hindlimb

unloaded rats [105], the effect of sex was poorly investigated in spaceflight animals, with no data available for female rats or primates, and only some mouse studies reporting changes in females.

In humans, significant association between bone loss and the bone position relative to the gravitational vector was identified [5,98]. Although it is more difficult to account for an equivalent gravitational vector in rodents, we attempted to assess the regional difference in bones of rodents assuming their quadrupedal movement. We have found that similar to humans, in rodents distal skeletal regions exhibited the trend of an increased trabecular bone deficits compared to axial skeletal regions. Furthermore, in two mouse studies that measured total BV/TV of the calvariae[60,64] an increase was reported. These data suggest that local factors, including microgravity-induced redistribution of body fluid [110], or change in mechanical environment [111] likely contribute to poor bone health.

We demonstrate that spaceflight is associated with strong inhibition of bone formation in rats, mice and primates, while osteoclast indices were not affected in rats, variably affected in mice, and not reported in primates. In contrast, in astronauts, resorption was found to raise rapidly, reaching a sustained 2-fold increase for the duration of the spaceflight, while formation was decreased or unchanged in the beginning of the mission after which it gradually increased over time[5]. However, the direct comparison between animal and human data is difficult due to important methodological differences in data acquisition. While in animals bone turnover is predominantly assessed histologically at the end of the space mission, in humans, biochemical markers of bone formation and resorption are measured in serum or urine, allowing for assessment during the spaceflight mission. Importantly, most histological markers only indicate the change in bone cell numbers, while circulating markers reflect both changes in number and function of bone cells. Nevertheless, we believe that the data conclusively indicate that bone formation is inhibited

in animals during spaceflight, because indices related to osteoblast numbers (osteoblast numbers and surface), and histomorphometric measures of osteoblast function (osteoid surface and thickness, mineral apposition rate and bone formation rate), were lower in spaceflight rodents or primates. In contrast, bone resorption data for spaceflight animals is less consistent and more difficult to compare to humans. Osteoclast numbers or surface uniformly did not change in rats, while in mice missions STS-131 and Bion M1 reported strong increases in osteoclast number and surface, but mission STS-108 demonstrated no change. Osteoclast function was assessed using circulating markers in two missions: in mission STS-108, that reported no change in osteoclast number, circulating TRAP5b was higher [{57}]; and in mission STS-118, a 13 days mission with mice for which no histological osteoclast data is available, circulating TRAP5b did not change [{58}]. Among articles that report a decrease in bone formation and not resorption during spaceflight in rats [{36,42,43,49}], no hypotheses were offered to explain this phenomenon. However, we speculate that the young age and associated rapid bone growth of the included rats may have been a contributing factor. Thus, although the data suggest that there may be a difference in the response of bone cells to microgravity between rodents and humans, and/or between mice and rats, we are limited by different nature of measurements in animals and humans, and a small sample size for mice. Therefore, more experiments assessing both bone cell numbers and function, especially for osteoclasts, are required to understand the spaceflight-induced changes in bone turnover.

This study for the first time has attempted to quantitatively integrate nearly 50 years of bone research in spacefaring animals. The limitations of this analysis included *i)* the differences in design of experiments in individual missions, *ii)* inconsistent reporting, and *iii)* the need to meta-analytically combine data performed using different protocols over a large time interval. Experimental design of individual missions evolved with time, however notably, there was little

data from spaceflights longer that 30 days, and there were no inflight measures of bone turnover or quality, which prevented us from assessing the long-term and dynamic effects of microgravity to animal bone. Of specific importance for animal experiments, is the design of the ground control, which aimed to model the parameters of spaceflight other than microgravity, was vastly different between missions. While this resulted in a limitation of comparing experimental groups to very different controls, it also allowed us to perform a preliminary assessment of relative effects of stressors associated with spaceflight other than microgravity. Since the extent of modeling the stressors in ground control groups was not associated with differential bone deficits, we concluded that the microgravity is the main driver of these changes. The most rigorous control for the specific effects of microgravity was in-space artificial gravity, which was performed during three missions, Cosmos 936, SpaceX CRS-9 and CRS-12. When the in-flight 1g group was used as a "ground control", the effect sizes for bone changes were not smaller than in missions with ground controls of lower fidelity. In addition, for Cosmos 936 which also had an associated vivarium control group, ground to vivarium control effect sizes and 95% CI were not significantly different from other ground to vivarium control comparisons, altogether suggesting that microgravity is the driving factor for bone loss in space. Nevertheless, we did identify several parameters, including trabecular thickness, and osteoclast surface and number that appear to be specifically affected in ground control compared to vivarium control groups, suggesting that other spaceflight associated factors may contribute to those changes. The second set of limitations was relevant to data reporting in the manuscripts. In multiple instances inconsistent reporting of animal treatment between papers reporting the same mission was observed. Rodent death was not uncommon during spaceflight, however it was infrequently reported, even though it reflects the stressful conditions during a particular mission, which then could not be accounted for in our analysis. Specifications regarding

bone surfaces analyzed in addition to control and spaceflight animal treatment/housing were often vague making categorizing for subgroup analysis difficult. In addition, degree of movement, which has a potential to affect bone health [4], was never reported in the included articles in rodents. This represents a significant shortcoming in reporting of the outcomes of animal experiments in space, since for several missions animal behavior data has been collected[112]. Therefore, similar to human studies [113], improving reporting practices of animal experiments by the Space Life Sciences Programs is critically important. The third set of limitations was related to performing meta-analysis on studies completed over a considerable interval of time with vastly different protocols. This resulted in our dataset being moderate to highly heterogeneous for 15 out of the 17 parameters. While we attempted to identify all possible factors that may account for the high degree of variation in our results, no single factor accounted for a major amount of variation in any of the measured outcomes. Since our analysis indicates low publication bias, high heterogeneity likely reflects the multifactorial nature of microgravity-induced bone changes, which can only be investigated through the analysis of larger datasets.

9. Conclusion

In conclusion, we demonstrate that meta-analysis of animal spaceflight data provides important additional information regarding the effect of microgravity on animal physiology, in particular allowing to perform comparative studies, which otherwise are financially and technologically challenging. Our studies on animals and humans [5] demonstrate that microgravity-induced deterioration of bone health is a complex phenomenon, with strong regional and temporal differences, as well as potentially different mechanisms of adaptation in different species. In the future, longer missions with planned in flight data collection are needed to understand the dynamics of changes in bone tissue and especially bone turnover, which appear to be different between humans and rodents. For nonhuman animals in particular, it is also important to relate the changes in bone to the movement patterns and activity, which are rarely provided in bone health focused studies. The quantitative estimates of spaceflight-related changes in bone health provided by our study will inform future studies and help in determining the underlying mechanisms of observed effects.

10. References

1 NASA. Artemis Plan: NASA's Lunar Exploration Program Overview. 1-74 (2020).

2 NASA. *NASA's Journey to Mars*, <https://www.nasa.gov/content/nasas-journey-to-mars> (2014).

3 Mars One. *Current Mission Status*, <http://www.mars-one.com/about-mars-one/current-mission-status> (2016).

4 Vico, L. & Hargens, A. Skeletal changes during and after spaceflight. *Nat Rev Rheumatol* **14**, 229-245 (2018).

5 Stavnichuk, M., Mikolajewicz, N., Corlett, T., Morris, M. & Komarova, S. V. A systematic review and meta-analysis of bone loss in space travelers. *NPJ Microgravity* **6**, 13 (2020).

6 Gray, T. *A Brief History of Animals in Space*, <https://history.nasa.gov/animals.html> (2014).

7 Morey-Holton, E. R., Hill, E. L. & Souza, K. A. Animals and spaceflight: from survival to understanding. *J Musculoskelet Neuronal Interact* **7**, 17-25 (2007).

8 Pielke, R. & Byerly, R. Shuttle programme lifetime cost. *Nature* **472**, 38-38 (2011).

9 Norberg, C. *Human Spaceflight and Exploration*. (2013).

10 Williams, D. R. & Turnock, M. Human space exploration the next fifty years. *McGill Journal of Medicine* **13**, 76-76 (2011).

11 Medicine, I. o. *Health Standards for Long Duration and Exploration Spaceflight: Ethics Principles, Responsibilities, and Decision Framework*. (The National Academies Press, 2014).

12 Hargens, A. R. & Watenpaugh, D. E. Cardiovascular adaptation to spaceflight. *Med Sci Sports Exerc* **28**, 977-982 (1996).

13 Ronca, A. E., Souza, K. A. & Mains, R. C. Translational Cell & Animal Research in Space: Ames Research Center, 1965 - 2011. (NASA Special Publication, 2015).

14 Grigor'ev, A. I. & Il'in, E. A. Animals in space. *Her. Russ. Acad. Sci.* **77**, 541-549 (2007).

15 Monier-Faugere, M.-C., Chris Langub, M. & Malluche, H. H. in *Metabolic Bone Disease and Clinically Related Disorders* (eds Louis V. Avioli & Stephen M. Krane) 237-280e (Academic Press, 1998).

16 Seeman, E. in *Principles of Bone Biology* (eds John P. Bilezikian, T. John Martin, Thomas L. Clemens, & Clifford J. Rosen) 245-274 (Academic Press, 2020).

17 Boskey, A. L. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *BoneKEy Rep.* **2**, 447-447 (2013).

18 Hof, R. J. v. t. & Dall'Ara, E. in *Bone Research Protocols* (ed Aymen I. Idris) 507-531 (Springer New York, 2019).

19 Karner, C. M. & Hilton, M. J. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 12-19 (2018).

20 Breeland, G., Sinkler, M. A. & Menezes, R. G. (StatPearls Publishing, 2021).

21 Jilka, R. L. The relevance of mouse models for investigating age-related bone loss in humans. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**, 1209-1217 (2013).

22 Tamma, R., Carbone, C. & Colucci, S. in *Imaging of Prosthetic Joints: A Combined Radiological and Clinical Perspective* (eds Carlina V. Albanese & Carlo Faletti) 15-25 (Springer Milan, 2014).

23 Murshed, M. Mechanism of Bone Mineralization. *Cold Spring Harb. Perspect. Med.* **8**, a031229 (2018).

24 Boskey, A. L. & Robey, P. G. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 84-92 (2018).

25 Fuchs, R. K., Warden, S. J. & Turner, C. H. in *Bone Repair Biomaterials* (eds Josep A. Planell, Serena M. Best, Damien Lacroix, & Antonio Merolli) 25-68 (Woodhead Publishing, 2009).

26 Burdan, F. *et al.* Morphology and physiology of the epiphyseal growth plate. *Folia Histochem. Cytobiol.* **47**, 5-16 (2009).

27 Hasegawa, T. Ultrastructure and biological function of matrix vesicles in bone mineralization. *Histochem. Cell Biol.* **149**, 289-304 (2018).

28 Takayanagi, H. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 46-53 (2018).

29 Bonewald, L. F. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 38-45 (2018).

30 Bullock, W. A., Plotkin, L. I., Robling, A. G. & Pavalko, F. M. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 75-83 (2018).

31 Bradley, E. W., Westendorf, J. J., van Wijnen, A. J. & Dudakovic, A. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 31-37 (2018).

32 Stanley, T. D. & Doucouliagos, H. Neither fixed nor random: weighted least squares meta-analysis. *Stat Med* **34**, 2116-2127 (2015).

33 Hedges, L. V. A random effects model for effect sizes. *Psychol Bull* **93**, 388-395 (1983).

34 Asling, C. W. Histological Studies on Tibial Bone of Rats in the 1975 Cosmos-782 Flight: I. Endochondral Osteogenesis; Medullary Bone Turnover. Report No. TM-78525, 276-290 (NASA, 1978).

35 Morey, E. R. & Baylink, D. J. Inhibition of bone formation during space flight. *Science* **201**, 1138-1141 (1978).

36 Morey-Holton, E., Turner, R. T. & Baylink, D. J. Quantitative Analysis of Selected Bone Parameters. Report No. TM-78526, 135-178 (NASA, 1978).

37 Judy, M. M. Quantitative Analysis of Selected Bone Parameters: Supplement 3A: Trabecular Spacing and Orientation in the Long Bones. Report No. TM-81289, 177-198 (NASA, 1981).

38 Wronski, T. J. & Morey-Holton, E. Quantitative Analysis of Selected Bone Parameters. . Report No. TM-81289, 101-125 (NASA, 1981).

39 Jee, W. S., Wronski, T. J., Morey, E. R. & Kimmel, D. B. Effects of spaceflight on trabecular bone in rats. *Am. J. Physiol.* **244**, R310-314 (1983).

40 Rogacheva, I. V., Stupakov, G. P., Volozhin, A. I., Pavlova, M. N. & Poliakov, A. N. [Characteristics of bone tissue of rats after flight aboard biosputnik Kosmos-1129]. *Kosm Biol Aviakosm Med.* **18**, 39-44 (1984).

41 Kaplanskii, A. S., Durnova, G. N., Sakharova, Z. F. & Il'ina-Kakueva, E. I. [Histomorphometric analysis of the bones of rats on board the Kosmos 1667 biosatellite]. *Kosm Biol Aviakosm Med.* **21**, 25-31 (1987).

42 Vico, L. *et al.* Trabecular bone remodeling after seven days of weightlessness exposure (BIOCOSMOS 1667). *Am. J. Physiol.* **255**, R243-247 (1988).

43 Wronski, T. J., Morey-Holton, E. R., Doty, S. B., Maese, A. C. & Walsh, C. C. Histomorphometric analysis of rat skeleton following spaceflight. *Am. J. Physiol.* **252**, R252-255 (1987).

44 Doty, S., Morey-Holton, E., Durnova, G. & Kaplansky, A. Cosmos 1887: Morphology, histochemistry, and vasculature of the growing rat tibia. *FASEB J.* **4**, 16-23 (1990).

45 Vailas, A. C. *et al.* Effects of spaceflight on rat humerus geometry, biomechanics, and biochemistry. *FASEB J.* **4**, 47-54 (1990).

46 Zerath, E. *et al.* Effets osseux de 13 jours de microgravité chez le rat. Résultats de BIOCOSMOS 1887. *Trav. Sci.* **11**, 233-234 (1990).

47 Vailas, A. C. *et al.* Adaptation of young adult rat cortical bone to 14 days of spaceflight. *J. Appl. Physiol.* **73**, 4S-9S (1992).

48 Vico, L., Bourrin, S., Genty, C., Palle, S. & Alexandre, C. Histomorphometric analyses of cancellous bone from COSMOS 2044 rats. *J. Appl. Physiol.* **75**, 2203-2208 (1993).

49 Turner, R. T., Evans, G. L. & Wakley, G. K. Spaceflight results in depressed cancellous bone formation in rat humeri. *Aviat. Space Environ. Med.* **66**, 770-774 (1995).

50 Westerlind, K. C. & Turner, R. T. The skeletal effects of spaceflight in growing rats: Tissue-specific alterations in mrna levels for TGF-β. *J. Bone Miner. Res.* **10**, 843-848 (1995).

51 Zerath, E. *et al.* Effects of spaceflight and recovery on rat humeri and vertebrae: histological and cell culture studies. *J. Appl. Physiol.* **81**, 164-171 (1996).

52 Lafage-Proust, M. H. *et al.* Space-related bone mineral redistribution and lack of bone mass recovery after reambulation in young rats. *Am. J. Physiol.* **274**, R324-334 (1998).

53 Bateman, T. A. *et al.* Histomorphometric, physical, and mechanical effects of spaceflight and insulin-like growth factor-I on rat long bones. *Bone* **23**, 527-535 (1998).

54 Wronski, T. J. *et al.* Lack of effect of spaceflight on bone mass and bone formation in group-housed rats. *J. Appl. Physiol.* **85**, 279-285 (1998).

55 Zerath, E. *et al.* Spaceflight inhibits bone formation independent of corticosteroid status in growing rats. *J. Bone Miner. Res.* **15**, 1310-1320 (2000).

56 Vajda, E. G., Wronski, T. J., Halloran, B. P., Bachus, K. N. & Miller, S. C. Spaceflight alters bone mechanics and modeling drifts in growing rats. *Aviat. Space Environ. Med.* **72**, 720-726 (2001).

57 Lloyd, S. A. *et al.* Osteoprotegerin is an effective countermeasure for spaceflight-induced bone loss in mice. *Bone* **81**, 562-572 (2015).

58 Ortega, A. M. *et al.* in *ASME 2013 Summer Bioengineering Conference* Vol. 1A: Abdominal Aortic Aneurysms; Active and Reactive Soft Matter; Atherosclerosis; BioFluid Mechanics; Education; Biotransport Phenomena; Bone, Joint and Spine Mechanics; Brain Injury; Cardiac Mechanics; Cardiovascular Devices, Fluids and Imaging; Cartilage and Disc Mechanics; Cell and Tissue Engineering; Cerebral Aneurysms; Computational Biofluid Dynamics; Device Design, Human Dynamics, and Rehabilitation; Drug Delivery and Disease Treatment; Engineered Cellular Environments (Sunriver, Oregon, USA, 2013).

59 Blaber, E. A. *et al.* Microgravity induces pelvic bone loss through osteoclastic activity, osteocytic osteolysis, and osteoblastic cell cycle inhibition by CDKN1a/p21. *PLoS One* **8**, e61372 (2013).

60 Zhang, B., Cory, E., Bhattacharya, R., Sah, R. & Hargens, A. R. Fifteen days of microgravity causes growth in calvaria of mice. *Bone* **56**, 290-295 (2013).

61 Blaber, E. A. *et al.* Mechanical unloading of bone in microgravity reduces mesenchymal and hematopoietic stem cell-mediated tissue regeneration. *Stem Cell Res.* **13**, 181-201 (2014).

62 Berg-Johansen, B. *et al.* Spaceflight-induced bone loss alters failure mode and reduces bending strength in murine spinal segments. *J. Orthop. Res.* **34**, 48-57 (2016).

63 Macaulay, T. R., Siamwala, J. H., Hargens, A. R. & Macias, B. R. Thirty days of spaceflight does not alter murine calvariae structure despite increased Sost expression. *Bone Rep.* **7**, 57-62 (2017).

64 Gerbaix, M. *et al.* One-month spaceflight compromises the bone microstructure, tissue-level mechanical properties, osteocyte survival and lacunae volume in mature mice skeletons. *Sci. Rep.* **7**, 2659 (2017).

65 Gerbaix, M. *et al.* Eight Days of Earth Reambulation Worsen Bone Loss Induced by 1-Month Spaceflight in the Major Weight-Bearing Ankle Bones of Mature Mice. *Front. Physiol.* **9**, 746 (2018).

66 Shiba, D. *et al.* Development of new experimental platform 'MARS'-Multiple Artificial-gravity Research System-to elucidate the impacts of micro/partial gravity on mice. *Sci. Rep.* **7**, 10837 (2017).

67 Maupin, K. A. *et al.* Skeletal adaptations in young male mice after 4 weeks aboard the International Space Station. *NPJ Microgravity* **5**, 21 (2019).

68 Tominari, T. *et al.* Hypergravity and microgravity exhibited reversal effects on the bone and muscle mass in mice. *Sci. Rep.* **9**, 6614 (2019).

69 Cann, C. E., Patterson-Buckendahl, P. & Adachi, R. R. Calcium metabolism and correlated endocrine measurements in primates during Cosmos '83. Report No. TM-88223, 129-144 (NASA, 1986).

70 Cann, C., Rakhmanov, A. & Karolkov, V. Analysis of Radiographs and Biosamples From Primate Studies. Report No. TM-102254, 513-519 (NASA, 1990).

71 Zerath, E. *et al.* Effects of spaceflight on bone mineralization in the rhesus monkey. *J. Appl. Physiol.* **81**, 194-200 (1996).

72 Zérath, E. *et al.* Spaceflight affects bone formation in rhesus monkeys: a histological and cell culture study. *J. Appl. Physiol.* **93**, 1047-1056 (2002).

73 Zérath, E., Holy, X., Malouvier, A., Caissard, J. C. & Noguès, C. Rat and monkey bone study in the Biocosmos 2044 space experiment. *Physiologist* **34**, S194-195 (1991).

74 Turner, R. T., Morey, E. R., Liu, C. & Baylink, D. J. Altered bone turnover during spaceflight. *Physiologist* **22**, S73-74 (1979).

75 Wronski, T. J., Morey-Holton, E. & Jee, W. S. Cosmos 1129: spaceflight and bone changes. *Physiologist* **23**, S79-82 (1980).

76 Jee, W. S. S., Kimmel, D. B., Smith, C. & Dell, R. B. Quantitative Analysis of Selected Bone Parameters: Supplement 2: Bone Elongation Rate and Bone Mass in Metaphysis of Long Bones. Report No. TM-81289, 149-175 (NASA, 1981).

77 Spengler, D. M., Morey, E. R., Carter, D. R., Turner, R. T. & Baylink, D. J. Effects of Spaceflight on Structural and Material Strength of Growing Bone. *Exp. Biol. Med. (Maywood)* **174**, 224-228 (1983).

78 Wronski, T. J. & Morey, E. R. Effect of spaceflight on periosteal bone formation in rats. *Am. J. Physiol.* **244**, R305-309 (1983).

79 Wronski, T. J. & Morey, E. R. Alterations in calcium homeostasis and bone during actual and simulated space flight. *Med Sci Sports Exerc* **15**, 410-414 (1983).

80 Doty, S. B. Morphologic and histochemical studies of bone cells from SL-3 rats. *Physiologist* **28**, S225-226 (1985).

81 Vico, L. *et al.* Effects of weightlessness on bone mass and osteoclast number in pregnant rats after a five-day spaceflight (COSMOS 1514). *Bone* **8**, 95-103 (1987).

82 Holton, E., Berretta, D., Doty, S., Roberts, W. & Garetto, L. Gravity and Skeletal Growth: I. Gravity and Skeletal Growth. Report No. TM-102254, 113-122 (NASA, 1990).

83 Morey-Holton, E. R. & Arnaud, S. B. Skeletal responses to spaceflight. *Adv. Space Biol. Med.* **1**, 37-69 (1991).

84 Rakhmanov, A. S. *et al.* [The state of bone tissue in monkeys in experiments in the Cosmos-1887 biosatellite]. *Kosm Biol Aviakosm Med.* **25**, 42-44 (1991).

85 Vico, L., Novikov, V. E., Very, J. M. & Alexandre, C. Bone histomorphometric comparison of rat tibial metaphysis after 7-day tail suspension vs. 7-day spaceflight. *Aviat. Space Environ. Med.* **62**, 26-31 (1991).

86 Kaplansky, A. S., Durnova, G. N., Burkovskaya, T. E. & Vorotnikova, E. V. The effect of microgravity on bone fracture healing in rats flown on Cosmos-2044. *Physiologist* **34**, S196-199 (1991).

87 Doty, S. B., Morey-Holton, E. R., Durnova, G. N. & Kaplansky, A. S. Morphological studies of bone and tendon. *J. Appl. Physiol.* **73**, S10-S13 (1992).

88 Kirchen, M. E. *et al.* Effects of microgravity on bone healing in a rat fibular osteotomy model. *Clin. Orthop. Relat. Res.*, 231-242 (1995).

89 Durnova, G., Kaplanskii, A., Morey-Holton, E. & Vorobéva, V. Investigation of tibial bones of the rats exposed on board 'Spacelab-2': Histomorphometric analysis. *Kosm Biol Aviakosm Med.* **30**, 21-26 (1996).

90 Cavolina, J. *et al.* The Effects of Orbital Spaceflight on Bone Histomorphometry and Messenger Ribonucleic Acid Levels for Bone Matrix Proteins and Skeletal Signaling Peptides in Ovariectomized Growing Rats 1. *Endocrinology* **138**, 1567-1576 (1997).

91 Zerath, E. *et al.* Effects of Bion 11 14-day space flight on monkey iliac bone. *J .Gravit. Physiol.* **7**, S155-156 (2000).

92 Doty, S. B. Space Flight and Bone Formation. *Materwiss Werksttech* **35**, 951-961 (2004).

93 Johnson, R. B. *et al.* Effects of spaceflight on the attachment of tendons to bone in the hindlimb of the pregnant rat. *Anat. Rec.* **282**, 147-156 (2005).

94 Tavella, S. *et al.* Bone turnover in wild type and pleiotrophin-transgenic mice housed for three months in the International Space Station (ISS). *PLoS One* **7**, e33179 (2012).

95 Keune, J. A., Philbrick, K. A., Branscum, A. J., Iwaniec, U. T. & Turner, R. T. Spaceflight-induced vertebral bone loss in ovariectomized rats is associated with increased bone marrow adiposity and no change in bone formation. *NPJ Microgravity* **2**, 16016 (2016).

96 Dadwal, U. C. *et al.* The effects of spaceflight and fracture healing on distant skeletal sites. *Sci. Rep.* **9**, 11419 (2019).

97 Mikolajewicz, N. & Komarova, S. V. Meta-Analytic Methodology for Basic Research: A Practical Guide. *Front. Physiol.* **10** (2019).

98 Oganov, V. S. *et al.* [Reactions of the human bone system in space flight: phenomenology]. *Kosm Biol Aviakosm Med.* **39**, 3-9 (2005).

99 Vico, L. *et al.* Cortical and Trabecular Bone Microstructure Did Not Recover at Weight-Bearing Skeletal Sites and Progressively Deteriorated at Non-Weight-Bearing Sites During the Year Following International Space Station Missions. *J. Bone Miner. Res.* **32**, 2010-2021 (2017).

100 Lang, T. *et al.* Cortical and Trabecular Bone Mineral Loss From the Spine and Hip in Long-Duration Spaceflight. *J. Bone Miner. Res.* **19**, 1006-1012 (2004).

101 Cunningham, H. C. *et al.* Age-dependent bone loss and recovery during hindlimb unloading and subsequent reloading in rats. *BMC Musculoskelet. Disord.* **19**, 223 (2018).

102 Ishijima, M. *et al.* Enhancement of Osteoclastic Bone Resorption and Suppression of Osteoblastic Bone Formation in Response to Reduced Mechanical Stress Do Not Occur in the Absence of Osteopontin. *J. Exp. Med.* **193**, 399-404 (2001).

103 Morse, A. *et al.* Mechanical load increases in bone formation via a sclerostin-independent pathway. *J. Bone Miner. Res.* **29**, 2456-2467 (2014).

104 Basso, N., Bellows, C. G. & Heersche, J. N. M. Effect of simulated weightlessness on osteoprogenitor cell number and proliferation in young and adult rats. *Bone* **36**, 173-183 (2005).

105 David, V. *et al.* Two-week longitudinal survey of bone architecture alteration in the hindlimb-unloaded rat model of bone loss: sex differences. *Am. J. Physiol. Endocrinol. Metab.* **290**, E440-E447 (2006).

106 Bloomfield, S. A., Allen, M. R., Hogan, H. A. & Delp, M. D. Site- and compartment-specific changes in bone with hindlimb unloading in mature adult rats. *Bone* **31**, 149-157 (2002).

107 Chen, M. M. *et al.* Adaptation of cancellous bone to aging and immobilization in growing rats. *Anat. Rec.* **234**, 317-334 (1992).

108 Barou, O., Palle, S., Vico, L., Alexandre, C. & Lafage-Proust, M. H. Hindlimb unloading in rat decreases preosteoblast proliferation assessed in vivo with BrdU incorporation. *Am. J. Physiol. Endocrinol. Metab.* **274**, E108-E114 (1998).

109 Shen, V. *et al.* Short-term immobilization-induced cancellous bone loss is limited to regions undergoing high turnover and/or modeling in mature rats. *Bone* **21**, 71-78 (1997).

110 Marenzana, M. & Arnett, T. R. The Key Role of the Blood Supply to Bone. *Bone Res.* **1**, 203-215 (2013).

111 Leblanc, A. D., Schneider, V. S., Evans, H. J., Engelbretson, D. A. & Krebs, J. M. Bone mineral loss and recovery after 17 weeks of bed rest. *J. Bone Miner. Res.* **5**, 843-850 (1990).

112 Ronca, A. E. *et al.* Behavior of mice aboard the International Space Station. *Sci. Rep.* **9**, 4717 (2019).

113 Corlett, T., Stavnichuk, M. & Komarova, S. V. Population analysis of space travelers. *Life Sci Space Res* **27**, 1-5 (2020).

Supplementary Information for the manuscript "Bone health in spacefaring rodents and primates: systematic review and meta-analysis" by Jingyan Fu, Matthew Goldsmith, Sequoia D. Crooks, Sean F. Condon, Martin Morris, Svetlana V. Komarova

Supplementary notes

Supplementary note 1. Search Strategy

1. Animal*.mp or exp Mammals/ or mammal.mp.
2. exp Hominidae/ or Hominidae.mp. or exp Pan troglodytes/ or exp Pongidae/ or chimpanzee.mp. or exp Hylobates/ or gibbon.mp. or ape.mp or great ape.mp
3. exp Cercopithecidae/ or Monkey.mp. or Macaca.mp. or Macaque.mp. or exp Macaca mulatta/ or rhesus monkey.mp. or exp Macaca fascicularis/ or cynomolgus monkey.mp. or crab eating macaque.mp. or exp Macaca nemestrina/ or pig-tailed macaque.mp. or pigtail macaque.mp
4. exp Saimiri/ or squirrel monkey.mp.
5. exp Cebus/ or Cebus paella.mp or tufted capuchin.mp.
6. exp Rodentia/ or rodent.mp. or exp Muridae/or exp Cricetidae/ or exp Rats/ or rat*.mp. or rattus.mp. or exp Mice/ or mouse.mp. or mice.mp or mus.mp or exp Gerbillinae/ or Meriones.mp. or Gerbil*.mp. or exp Caviidae/ or exp Guinea Pigs/ or Guinea pig.mp.
7. exp Lagomorph*/ or exp Leporidae/ or exp Rabbits/ or rabbit*.mp.
8. exp Carnivor*/ or exp Canidae/ or exp Dogs/ or dog*.mp. or Canis.mp. or Canidae.mp. or exp Felidae/ or Cat.mp. or felis.mp.
9. exp Reptiles/ or exp Chelon*/ or exp Turtles/ or Tortoise.mp. or Turtle.mp. or Reptilia.mp. or exp Lizards/ or Gecko.mp. or Sauria.mp.
10. exp Birds/ or exp Chickens/ or exp Quail/ or exp Galliformes/ or exp Coturnix/ or Bird.mp. or chick*.mp. or quail.mp. or galliform*.mp. or Coturnix.mp. or Gallus.mp.
11. exp Amphibians/ or Amphibi*.mp. or exp Anura/ or Salientia.mp. or anura.mp or exp Ranidae/ or frog.mp. or rana.mp. or exp Bufonidae/ or toad.mp. or Bufo.mp or exp Xenopus/ or Xenopus.mp. or exp Urodela/ or Urodela.mp. or Newt.mp. or Salamander.mp. or Urodel*.mp.
12. exp Pisces/ or exp Osteichthyes/ or Fish.mp. or exp Fish/ or Oryzias.mp. or Medaka.mp. or Zebrafish.mp. or Danio.mp. or Xiphophorus.mp. or Mummichog.mp. or Cyprinus.mp.
13. or/1-12
14. exp Space Flight/
15. exp Weightlessness/
16. exp Extraterrestrial Environment/
17. ((soyuz* or apollo* or gemini or "international space station" or saluyt or skylab or shenzhou or voskhod or euromir or NASA or voskhod or tiangong or mir or mercury or shuttle or ISS or ESA or CNSA or NASDA or Sputnik* or Atlas or Biosatellite or Zond or Bion or Spacelab or Foton or Genesis or SpaceX) and (space* or orbit* or station* or mission*)).ti,ab,kf.
18. (space adj5 (flight* or travel* or explor* or outer)).ti,ab,kw.
19. or/14-18
20. exp "Bone and Bones"/ or exp Bone Diseases/ or exp Osteogenesis/ or exp Bone Density/ or exp Bone Remodeling/
21. (bone* or osseo* or osteo* or skelet* or musculoskelet*).ti,ab,kw.
22. (skeletal or musculoskeletal or tarsal* or metatarsal* or calcaneus or talus or femur or fibula or patella or fibia or humerus or radius or ulna or clavicle or acromion or glenoid or diaphyses or epiphyses or hyoid or sesamoid or cranium or cranial or occipital or basilar or foramen or basicranium or sphenoid or mastoid or petrous or odontoid or parietal or fossa or skull or spenoid

or mandible or maxilla or vomer or zygoma or vertebra* or sacrum or rib or ribs or sternum or manubrium or coccyx).ti,ab,kw.
23. or/20-22
24. 13 and 19 and 23

Supplementary note 2. Quality score checklist for full text appraisal

(Total of 25)

1. Mission title & flight duration are clearly stated (1)
2. Clear indication of: (maximum of 4)
   sex (1), age (1), weight preflight (0.5), weight postflight (0.5), and sample size: n_{SF} (1) of spaceflight animals
3. Study contains the following control groups: (maximum 3 points)
   preflight or baseline control group (1), ground control group (1), vivarium control group (1)
4. Specify housing conditions of: (maximum 2 points)
   spaceflight group: group vs single housing (0.5) and specific habitat (0.5);
   ground control group: specific conditions in reference to spaceflight group (1)
5. Time of sacrifice/measurements for: (maximum 2 points)
   spaceflight group (1) and control group(s) (1)
6. All data was presented in a table (1) or in a graph form (0);
7. When averaged data are presented clearly show sample size (n) for each measurement (2)
8. Clearly indicate all measurement units (including type of data spread) (2)
9. Specific bone region from which measurements are taken is defined (1) and measurement techniques used are indicated (1)
10. Data regarding the following bone parameters shown: (maximum 3 points)
    trabecular bone parameters (1), cortical bone parameters (1), bone turnover parameters (1)
11. Accurately report appropriate data/units (3), or contains evidence of misreporting (0)

Supplementary Tables

Supplementary Table 1. PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	4,5
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	8,9
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	9
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	The study was not registered
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	16
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	16
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplemental information S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	16,Supplemental information S2
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	17
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	17, Table 1, S2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	21
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	22
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	18-20

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	21
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	21-22
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	23, Fig. 1A, Table S3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2, S4, S5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Fig. 2, S1, S2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig. 3-7, Tables S6-S18
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	28-34, Fig. 3-7, Table 3, S6-S18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Fig. 2, S1
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	37-38, Fig. 2, 8, S2-S7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	40-43
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	43-45
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	46
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	6

Supplementary Table 2. Alternative Terms used for included parameters

Parameters (Abbreviation)	Alternate Term
Trabecular Bone Measures	
1. Trabecular bone volume fraction (Tb.BV/TV)	- BV/TV [27,28,31] - Trabecular Bone Volume [15,18,19,27,28,31] - Cancellous Bone Volume [24,28,30] - Fractional Area of Mineralized Tissue [15]
2. Trabecular Number (Tb.N)	- Trabecular density [18]
3. Trabecular Thickness (Tb.Th)	N/A
4. Trabecular Separation (Tb.Sp)	N/A
5. Connectivity Density	N/A
6. Total BV/TV	- Total Bone Volume [27]
Cortical Bone Measures	
1. Marrow Area (Ma.Ar)	- Medullary Area [11,12,17,23,32]
2. Marrow Diameter (Ma.Dm)	- Medullary Diameter [21]
3. Cortical Bone Area (Ct.Ar)	- Cortical Plate Area [17] - Cortical Cross-sectional area [23]
4. Cortical Thickness (Ct.Th)	- Cortical Width [28]
Bone Turnover Measures	
Osteoblast Surface (Ob.S)	-Forming surface [12]
Osteoblast Number (N.Ob)	- Osteoblast number per bone perimeter (Ob.N/B.Pm) [19] - Number of osteoblasts per length of bone [15,19]
Osteoid Surface (OS/BS)	- Length of osteoid seam covering bone forming Surfaces [24,28] - percentage of bone area covered in osteoid [27]
Osteoid Thickness (O.Th)	- Thickness of osteoid seam [18] - width of osteoid surface [27]
Osteoclast Surface (Oc.S)	- Active Resorption Surface [18,24] - Fraction of bone surface length covered with Osteoclasts [18,24,27,28]
Osteoclast Number (N.Oc.)	- Osteoclast number per bone perimeter (N.Oc./B.Pm) [28] - Number of osteoclasts per length of bone [18,19,24,28]
Bone Formation Rate (BFR)	N/A
Mineral Apposition Rate (MAR)	- Calcification rate [49]

BV/TV = Bone volume/tissue volume

Supplementary Table 3. Removed Articles with Quantitative measures of bone health

Article Reference	Mission(s)	Species	Exclusion Reason
Turner 1979 et al. [50]	Cosmos 782 & 936	Rats	Only show relative changes, raw data present in Morey 1978 (Cosmos 782) and Morey-Holton 1978 (Cosmos 936)
Wronski 1980 et al. [51]	Cosmos 1129	Rats	Data present Wronski 1981 and Jee 1983
Jee 1981 et al. [52]	Cosmos 1129	Rats	Data present in Jee 1983
Spengler 1983 et al. [53]	Cosmos 936	Rats	Data present in Morey-Holton 1978
Wronski 1983a et al. [55]	Cosmos 782, 936, & 1129	Rats	Data present in Morey 1978 (Cosmos 782), Morey-Holton 1978 (Cosmos 936), and Wronski 1981 (Cosmos 1129)
Wronski 1983b et al. [54]	Cosmos 1129	Rats	Data present in Wronski 1981
Doty 1985 [56]	SpaceLab 3	Rats	Measure of osteoblast number did not have a defined location
Vico 1987 et al. [57]	Cosmos 1514	Rats	All spaceflight rodents were pregnant
Holton 1990 et al. [58]	Cosmos 1887	Rats	Data present in Doty 1990
Morey-Holton 1991 et al. [59]	Cosmos 936	Rats	Data present in Morey-Holton 1978
Rakhmanov 1991 et al. [60]	Cosmos 1887	Primates	Data present in Cann 1990
Vico 1991 et al. [61]	Cosmos 1667	Rats	Data present in Vico 1988
Kaplansky 1991 et al. [62]	Cosmos 2044	Rats	All spaceflight animals received bone fracture
Doty 1992 et al. [63]	Cosmos 2044	Rats	Measured number of "active" osteoblasts, not included due to vague definition of active
Kirchen 1995 et al. [64]	STS-29	Rats	Measure osteoclast number per arbitrary bone region
Durnova 1996 et al. [65]	STS-58	Rats	All data presented as averages with no measure of variation for any recorded parameter
Cavolina 1997 et al. [66]	STS-62	Rats	All spaceflight animals were ovariectomized
Zerath 2000b et al. [67]	Bion 11	Primates	Data presented better in Zerath 2002
Doty 2004 [68]	Cosmos 1129	Rats	Data presented in Wronski 1981
Johnson 2005 et al. [69]	STS-66	Rats	All spaceflight rodents were pregnant
Tavella 2012 et al. [70]	MDS	Mice	Only contain 3 spaceflight mice, all but 1 died before returning to Earth
Keune 2016 et al.[71]	STS-62	Rats	All spaceflight animals were ovariectomized
Dadwal 2019 et al. [72]	SpaceX CRS-10	Mice	Data present in Maupin 2019

Supplementary Table 4. Parameters including in meta-analysis

Year	Mission	Article References	Species	Tb.BV/TV	Tb.Th	Tb.N	Tb.Sp	Con.D	T.BV/TV	Ob.S	N.Ob	OS/BS	O.Th	Oc.S	N.Oc	Ma.A/D	Ct.Ar	Ct.Th	BFR	MAR
1975	Cosmos 782	**Asling 1978** [10]	**Rats**	✓	✓	✓														
		Morey 1978 et al. [11]														✓			✓	
1977	Cosmos 936	**Morey-Holton 1978 et al.** [12]	**Rats**							✓					✓	✓			✓	
1979	Cosmos 1129	**Judy 1981** [13]	**Rats**				✓													
		Wronski 1981 et al. [14]														✓				
		Jee 1983 et al. [15]		✓							✓									
		Rogacheva 1984 et al. [16]														✓		✓		
1983	Cosmos 1514	**Cann 1986 et al.** [45]	**Primates**															✓		
1985	Cosmos 1667	**Kaplanskii 1987 et al.** [17]	**Rats**	✓							✓				✓	✓	✓			
		Vico 1988 et al. [18]		✓	✓	✓	✓					✓	✓	✓	✓					
1985	SpaceLab 3	**Wronski 1987 et al.** [19]	**Rats**	✓						✓	✓			✓	✓				✓	
1987	Cosmos 1887	**Doty 1990 et al.** [20]	**Rats**													✓	✓			
		Vailas 1990 et al. [21]														✓	✓			
		Zerath 1990 et al. [22]		✓																
		Cann 1990 et al. [46]	**Primates**															✓		
1989	Cosmos 2044	**Zérath 1991 et al.** [49]	**Primates**	✓						✓										
			Rats																	✓
		Vailas 1992 et al. [23]														✓	✓			
		Vico 1993 et al. [24]		✓	✓	✓	✓			✓		✓		✓	✓					
1992	Bion 10	**Zerath 1996b et al.** [47]	**Primates**	✓	✓	✓	✓													✓
1992	STS-52	**Turner 1995 et al.** [25]	**Rats**	✓		✓	✓			✓		✓		✓	✓					
1992	STS-57	**Westerlind 1995 et al.** [26]	**Rats**	✓												✓	✓		✓	✓
1993	STS-58 (SLS-2)	**Zerath 1996a et al.** [27]	**Rats**	✓	✓	✓	✓		✓	✓		✓	✓	✓						
		Lafage-Proust 1998 et al. [28]		✓	✓	✓				✓		✓		✓	✓					
1996	Bion 11	**Zerath 2002 et al.** [48]	**Primates**	✓	✓	✓	✓					✓	✓	✓				✓	✓	✓
1996	STS-77	**Bateman 1998 et al.** [29]	**Rats**																	✓
1996	STS-78	**Wronski 1998 et al.** [30]	**Rats**	✓						✓				✓			✓	✓	✓	✓
		Zerath 2000a et al. [31]		✓	✓	✓	✓			✓			✓						✓	
		Vajda 2001 et al. [32]														✓	✓		✓	✓

Year	Mission	Article Reference	Species	Tb.BV/TV	Tb.Th	Tb.N	Tb.Sp	Con.D	T.BV/TV	Ob.S	N.Ob	OS/BS	O.Th	Oc.S	N.Oc	Ma.A/D	Ct.Ar	Ct.Th	BFR	MAR
2001	STS-108	**Lloyd 2015 et al.** [33]	**Mice**	✓	✓	✓	✓	✓		✓				✓				✓	✓	✓
2007	STS-118	**Ortega 2013 et al.** [34]	**Mice**	✓	✓	✓	✓	✓									✓			✓
2010	STS-131	**Blaber 2013 et al.** [37]	**Mice**						✓					✓	✓					
		Zhang 2013 et al. [36]							✓											
		Blaber 2014 et al. [37]		✓	✓	✓	✓	✓								✓	✓	✓		
2013	Bion M1	**Berg-Johansen 2016 et al.** [38]	**Mice**	✓	✓															
		Macaulay 2017 et al. [39]							✓									✓		
		Gerbaix 2017 et al. [40]		✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓		
		Gerbaix 2018 et al. [41]							✓									✓		
2016	SpaceX CRS-9	**Shiba 2017 et al.** [42]	**Mice**	✓																
2017	SpaceX CRS-10	**Maupin 2019 et al.** [43]	**Mice**	✓	✓	✓	✓		✓							✓	✓	✓		
2017	SpaceX CRS-12	**Tominari 2019 et al.** [44]	**Mice**	✓	✓	✓	✓													

Parameter abbreviations: Tb.BV/TV = trabecular BV/TV; Tb.Th = trabecular thickness; Tb.N = trabecular number; Tb.Sp = trabecular separation; Con.D = connective density; T.BV/TV = total BV/TV; Ob.S = osteoblast surface area; N.Ob = osteoblast number; OS/BS = osteoid surface/bone surface; O.Th = osteoid thickness; Oc.S = osteoclast surface area; N.Oc = osteoclast number; Ma.A/D = bone marrow area/diameter; Ct.A = cortical bone area; Ct.Th = cortical bone thickness; BFR = bone formation rate; MAR = mineral apposition rate.

Supplementary Table 5. Rodent study characteristics used for covariate analysis

Mission	Articles	Bones (Sub-sections)			Species	Strain	Sex	Age	SF Sacrifice Delay	Group House	Sham Op.	GC Cond. (scale 1-3)
		Region 1	Region 2	Region 3								
Cosmos 782	**Asling 1978**			Tibia (M)	Rats	Wistar	Male	9w	NR			3
	Morey 1978			Tibia (D)								
Cosmos 936	**Morey-Holton 1978**			Tibia (D)	Rats	Wistar	Male	9w	NR			3
Cosmos 1129	**Judy 1981**			Tibia (M)	Rats	Wistar	Male	11w 6d	7-11h			3
	Wronski 1981	Rib (NS)	Humerus (D)	Tibia (D)								
	Jee 1983		Humerus (M)	Tibia (M)								
	Rogacheva 1984		Femur (D)									
Cosmos 1667	**Kaplanskii 1987**	Vertebrae (L)	Pelvis (Ilium)	Tibia (D/M)	Rats	Wistar	Male	15w	4-8h 6h			2
	Vico 1988	Vertebrae (T8/L1)	Femur (M)	Tibia (M)								
SpaceLab3	**Wronski 1987**	Vertebra (L4)	Humerus (M)	Tibia (D)	Rats	Sprague-Dawley	Male	(S): 8w (L): 12w	11-17h			NR
Cosmos 1887	**Vailas 1990**		Humerus (D)		Rats	Wistar	Male	12w 6d	1d 18h	✓		3
	Doty 1990			Tibia (D)								
	Zerath 1990	Vertebrae (NS)	Humerus (M)									
Cosmos 2044	**Zerath 1991**	Vertebra (T9)	Humerus (M)		Rats	Wistar	Male	12w 5d	3-11h	✓	✓	3
	Vailas 1992		Humerus (D)									
	Vico 1993	Vertebrae (L2/T5)	Femur (M)	Tibia (E/M)								
STS-52	**Turner 1995**		Humerus (M)		Rats	Sprague-Dawley	Male	6w	2d 5h	✓		NR
STS-57	**Westerlind 1995**		Femur (D)	Tibia (M)	Rats	Fischer 344	Male	7-8w	5-8h	✓		2
STS-58 (SLS-2)	**Zerath 1996a**	Vertebrae (T9/C7)	Humerus (M)		Rats	Sprague-Dawley	Male	8w	4-6h			2
	Lafage-Proust 1998		Femur (M), Humerus (M)									

STS-77	**Bateman 1998**		Humerus (D)	Tibia (D)	Rats	Sprague-Dawley	Male	5w 5d	3-6h	✓		N/A
STS-78	**Wronski 1998**	Vertebra (L1)		Tibia (M/D)	Rats	Sprague-Dawley	Male	6w 3d	4-7h	✓	✓	1
	Zerath 2000a	Vertebra (T8)	Pelvis (Cotyloid)									
	Vajda 2001		Femur (D)									
STS-108	**Lloyd 2015**	Vertebra (L5)	Humerus (M) Femur (D)	Tibia (M)	Mice	C57BL/6	Female	9w 1d	3h 30m	✓		2
STS-118	**Ortega 2013**		Femur (M)	Tibia (M/D)	Mice	C57BL/6	Female	9w	3-6h	✓		NR
STS-131	**Blaber 2013**		Pelvis (Ischium), Femur (M)		Mice	C57BL/6J	Female	16w	2h	✓		1
	Zhang 2013	Calvaria										
	Blaber 2014		Femur (E/M)									
Bion M1	**Berg-Johansen 2016**	Vertebrae (C)			Mice	C57BL/6N	Male	19-20w	13-15h 13-24h	✓		2
	Macaulay 2017	Calvaria										
	Gerbaix 2017	Vertebrae (L1/L3/T12)	Femur (M/D)									
	Gerbaix 2018			Calcaneus, Navicular, Talus								
SpaceX CRS-9	**Shiba 2017**		Femur (prox)		Mice	C57BL/6J	Male	8w	NR			3
SpaceX CRS-10	**Maupin 2019**	Calvaria, Rib (10), Sternum, Vertebra (L4)	Humerus(M/D), Femur (M/D)	Tibia (M/D)	Mice	C57BL/6J	Male	9w	NR	✓	✓	2
SpaceX CRS-12	**Tominari 2019**		Humerus (prox)	Tibia (prox)	Mice	C57BL/6J	Male	9w	NR			3

Bones are organized by skeletal region (Region 1: bones of the head, vertebrae and thorax, Region 2: pelvis, humerus and femur, Region 3: tibia and ankle). Longbone sub-sections (epiphysis (E), metaphysis (M), or diaphysis (D)) are indicated. For vertebrae type (lumber (L), thoracic (T), or caudal (C)) and number are indicated. w = weeks, h = hours. m = minutes. NS = not specified. NR = not recorded. GC Cond. = Ground control conditions rated from 1 (poorest) to 3 (best) consideration to spaceflight associated conditions other than microgravity.

Supplementary Table 6. Trabecular Number

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	3.8	[-1.3,9.0]	*NA*	*NA*
	STS-118	13	12	-8.5	[-14.0,-3.1]	*NA*	*NA*
	STS-131	15	8	-6.0	[-10.8,-1.2]	*NA*	*NA*
	Bion M1 (2)	30	5/6	-10.2	[-22.6,2.3]	-9.2	[-18.7,0.2]
	SpaceX CRS-10	28	10	-0.5	[-17.9,16.9]	*NA*	*NA*
	SpaceX CRS-12	34	3	-43.7	[-71.7,-15.8]	*NA*	*NA*
Mice Overall				**-6.2**	**[-15.9,3.6]**	*NA*	*NA*
Rats	Cosmos 782	19.5	6	-24.7	[-42.7,-6.7]	8.7	[-8.5,26.0]
	Cosmos 1667	7	7	-22.0	[-43.8,-0.2]	*NA*	*NA*
	Cosmos 2044	14	5	-1.8	[-16.4,12.9]	-1.2	[-34.4,32.1]
	STS-52	10	6	8.4	[-14.8,31.6]	*NA*	*NA*
	STS-58	14	5	-11.1	[-29.7,7.5]	11.9	[-8.0,31.8]
	STS-78	17	6	-4.9	[-15.1,5.2]	6.8	[-4.2,17.9]
Rats Overall				**-9.9**	**[-19.2,-0.5]**	*NA*	*NA*
Rodents Overall				**-7.8**	**[-15.4,-0.1]**	**3.3**	**[-9.2,15.7]**
				I^2 = 68.8		**I^2 = 52.1**	

Supplementary Table 7. Trabecular Separation

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	-4.0	[-9.8,1.8]	*NA*	*NA*
	STS-118	13	12	10.4	[3.3,17.5]	*NA*	*NA*
	STS-131	15	8	2.6	[-3.4,8.6]	*NA*	*NA*
	Bion M1 (2)	30	5/6	11.4	[-3.7,26.5]	14.6	[1.7,27.5]
	SpaceX CRS-10	28	10	0.5	[-11.5,12.4]	*NA*	*NA*
	SpaceX CRS-12	34	3	110.9	[19.7,202.0]	*NA*	*NA*
Mice Overall				**10.4**	**[-13.8,34.7]**	*NA*	*NA*
Rats	Cosmos 1129*	18.5	7	3.4	[-17.9,24.7]	*NA*	*NA*
	Cosmos 1667	7	7	56.4	[10.6,102.1]	*NA*	*NA*
	Cosmos 2044	14	5	10.4	[-14.7,35.6]	7.2	[-42.5,56.9]
	STS-52	10	6	-16.8	[-52.9,19.4]	*NA*	*NA*
	STS-58	14	5	25.6	[-6.2,57.5]	-7.8	[-39.1,23.5]
	STS-78	17	6	7.0	[-6.8,20.7]	-5.7	[-19.2,7.9]
Rats Overall				**15.0**	**[-2.2,32.2]**	*NA*	*NA*
Rodents Overall				**12.4**	**[-4.8,29.6]**	**2.3**	**[-17.1,21.7]**
				I^2 = 80.8		**I^2 = 42.8**	

Supplementary Table 8. Connective Density

				SF vs GC	
Species	**Flight**	**Days**	**n_{SF}**	**ES (%)**	**95% CI**
Mice	STS-108	15	12	-10.2	[-41.8,21.4]
	STS-118	12	12	-45.0	[-67.3,-22.6]
	STS-131	13	8	13.5	[-6.4,33.3]
	Bion M1 (2)	30	5	-58.0	[-126.8,10.7]
Mice Overall				**-22.8**	**[-64.7,19.0]**
				I^2 = 83.5	

Supplementary Table 9. Total BV/TV

				SF vs GC	
Species	**Flight**	**Days**	**n_{SF}**	**ES (%)**	**95% CI**
Mice	STS-131	15	8	1.2	[-4.4,6.8]
	Bion M1 (1)	30	6	-5.0	[-27.5,17.5]
	Bion M1 (2)	30	5	-2.2	[-7.3,2.9]
	SpaceX CRS-10	30	10	1.9	[1.2,2.6]
Mice Overall				**-0.5**	**[-7.9,6.9]**
Rats	STS-58	14	5	-4.6	[-14.3,5.2]
Rodents Overall				**-1.1**	**[-9.1,6.9]**
				I^2 = 94.5	

Supplementary Table 10. Osteoblast Surface

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	-15.4	[-99.5,68.5]	*NA*	*NA*
	Bion M1 (2)	30	5/6	-35.2	[-82.1,11.7]	-11.5	[-63.1,40.1]
Rats	Cosmos 936	18.5	5	12.1	[-4.3,28.5]	*NA*	*NA*
	SpaceLab 3 (L)	7	4/5	-33.3	[-79.9,13.3]	*NA*	*NA*
	SpaceLab 3 (S)	7	6	-4.2	[-35.0,26.6]	*NA*	*NA*
	Cosmos 2044	14	5	-25.7	[-173.1,121.8]	-9.2	[-164.7,146.4]
	STS-58	14	5	-19.4	[-45.0,6.3]	-10.0	[-43.4,23.4]
	STS-78	17	6	-20.3	[-48.5,7.9]	1.5	[-31.1,34.1]
Rats Overall				**-15.2**	**[-56.4,25.9]**	*NA*	*NA*
Rodents Overall				**-17.4**	**[-54.3,19.5]**	**-6.9**	**[-20.2,6.4]**
				I^2 = 50.8		**I^2 = 0**	

Supplementary Table 11. Osteoblast Number

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Rats	Cosmos 1129	18.5	7	-36.0	[-66.5,-5.5]	0	[-21.0,21.0]
	Cosmos 1667	7	7	-16.4	[-23.7,-9.2]	-8.1	[-15.1,-1.2]
	SpaceLab 3 (L)	7	4/5	-33.9	[-78.6,10.8]	*NA*	*NA*
	SpaceLab 3 (S)	7	6	-8.9	[-42.2,24.4]	*NA*	*NA*
Rats Overall				**-23.2**	**[-51.0,4.7]**	**-4.1**	**[-9.0,0.8]**
				I^2 = 71.9		**I^2 = 31.7**	

Supplementary Table 12. Osteoid Surface

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	Bion M1 (2)	30	5/6	4.7	[-28.3,37.6]	-25.4	[-47.6,-3.1]
Rats	Cosmos 1667	7	7	-33.7	[-51.2,-16.2]	*NA*	*NA*
	Cosmos 2044	14	5	-18.2	[-37.6,1.2]	-29.3	[-44.9,-13.6]
	STS-52	10	6	-79.7	[-113.6,-45.9]	*NA*	*NA*
	STS-58	14	5	-11.0	[-40.8,18.8]	*NA*	*NA*
Rodents Overall				**-29.9**	**[-53.9,-5.8]**	**-27.1**	**[-29.8,-24.5]**
				I^2 = 74.5		**I^2 = 0**	

Supplementary Table 13. Osteoid Thickness

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Rats	Cosmos 1667	7	7	-16.7	[-27.0,-6.4]	*NA*	*NA*
	STS-58	14	5	-24.6	[-54.0,4.8]	-24.1	[-54.8,6.6]
	STS-78	17	6	-45.9	[-89.9,-2.0]	6.16	[-35.7,48.1]
Rats Overall				**-28.6**	**[-54.5,-2.7]**	**-7.6**	**[-40.2,25.0]**
				I^2 = 65.8		**I^2 = 34.3**	

Supplementary Table 14. Bone Marrow Area

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-131	15	8	35.9	[-4.5,76.2]	*NA*	*NA*
	Bion M1 (2)	30	5/6	-1.1	[-5.6,3.5]	5.8	[0.1,11.4]
	SpaceX CRS-10	28	10	-3.3	[-18.1,11.4]	*NA*	*NA*
Mice Overall				**10.8**	**[-0.2,21.8]**	*NA*	*NA*
Rats	Cosmos 782	19.5	6	-10.4	[-20.5,-0.4]	0.0	[-6.9,6.9]
	Cosmos 936	18.5	4	-8.5	[-17.4,0.0]	10.4	[-2.8,23.7]

	Cosmos 1129#	18.5	6	-3.3	[-30.1,23.4]	-24.5	[-36.8,-12.1]
	Cosmos 1667	7	7	0.0	[-6.6,6.6]	-11.1	[-15.5,-6.7]
	Cosmos 1887#	12.5	5	-7.9	[-31.8,15.9]	1.3	[-8.0,10.7]
	Cosmos 2044	14	5	-5.3	[-15.2,4.6]	2.7	[-2.7,8.1]
	STS-57	11	12	2.6	[-3.3,8.6]	1.0	[-1.5,3.5]
	STS-78	17	6	15.2	[-1.6,32.0]	15.2	[6.8,23.6]
Rats Overall				**-1.3**	**[-7.7,5.1]**	**-0.9**	**[-8.9,7.1]**
Rodents Overall				**2.4**	**[-4.3,9.1]**	**-0.2**	**[-8.9,8.6]**
				I^2 **= 21.1**		I^2 **=87.3**	

Supplementary Table 15. Cortical Bone Area

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-118	13	9/11	-3.8	[-7.1,-0.4]	*NA*	*NA*
	STS-131	15	8	-7.7	[-13.3,-2.1]	*NA*	*NA*
	Bion M1(2)	30	5/6	-5.7	[-13.7,2.3]	-4.3	[-10.4,1.7]
	SpaceX CRS-10	28	10	-6.4	[-16.9,4.1]	*NA*	*NA*
Mice Overall				**-5.9**	**[-8.8,-3.0]**	*NA*	*NA*
Rats	Cosmos 1887	12.5	5	-10.0	[-26.4,6.4]	5.8	[-12.6,24.3]
	Cosmos 2044	14	5	-7.9	[-12.8,-3.0]	-3.1	[-7.9,1.7]
	STS-57	11	12	-6.1	[-9.4,-2.9]	-3.9	[-6.7,-1.0]
	STS-78	17	6	-0.8	[-4.5,2.9]	3.6	[-3.8,11.0]
Rats Overall				**-6.0**	**[-9.4,-2.6]**	*NA*	*NA*
Rodents Overall				**-5.9**	**[-8.0,-3.8]**	**-1.1**	**[-6.2,4.0]**
				I^2 **=0**		I^2 **= 46.7**	

Supplementary Table 16. Cortical Thickness

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	-10.8	[-19.5,-2.1]	*NA*	*NA*
	STS-131	15	8	-1.4	[-3.6,0.8]	*NA*	*NA*
	Bion M1 (1)	30	6/7	3.0	[-6.5,12.5]	*NA*	*NA*
	Bion M1 (2)	30	5/6	-5.5	[-17.7,6.7]	-3.6	[-12.3,5.0]
	SpaceX CRS-10	28	10	-3.6	[-10.5,3.3]	*NA*	*NA*
Mice Overall				**-4.6**	**[-10.8,1.7]**	*NA*	*NA*
Rats	Cosmos 1129	18.5	6	-8.3	[-9.3,-7.3]	-7.2	[-19.9,5.5]
	STS-78	17	6	-1.7	[-6.4,2.9]	4.8	[-0.8,10.4]
Rodents Overall				**-4.7**	**[-13.7,4.4]**	**-2.0**	**[-11.8,7.7]**
				I^2 **= 90.7**		I^2 **= 68.6**	

Supplementary Table 17. Bone Formation Rate

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	-71.2	[-86,9,-55.5]	*NA*	*NA*
Rats	Cosmos 782	19.5	11/7	-40.5	[-53.1,-27.9]	1.3	[-9.9,12.4]
	Cosmos 936	18.5	10/8	-17.3	[-40.4,5.8]	9.6	[-16.3,35.4]
	Cosmos 1129	18.5	11	-33.7	[-46.9,-20.5]	-10.5	[-36.3,15.4]
	SpaceLab 3 (L)	7	4	-33.7	[-65.1,-2.3]	*NA*	*NA*
	STS-57	11	12	-10.6	[-33.6,12.4]	-12.8	[-30.2,4.6]
	STS-78	17	6	3.2	[-25.0,31.3]	1.7	[-27.0,30.4]
Rats Overall				**-22.8**	**[-39.1,-6.6]**	*NA*	*NA*
Rodents Overall				**-31.6**	**[-50.4,-12.8]**	**-9.2**	**[-24.3,5.8]**
				I^2 = 83.8		**I^2 = 65.4**	

Supplementary Table 18. Mineral Apposition Rate

				SF vs GC		GC vs VC	
Species	**Flight**	**Days**	**n_{SF}/n_{GC}**	**ES (%)**	**95% CI**	**ES (%)**	**95% CI**
Mice	STS-108	12	12	-30.4	[-46.0,-14.7]	*NA*	*NA*
	STS-118	13	9/11	-22.2	[-46.7,2.2]	*NA*	*NA*
Rats	STS-57	11	12	-2.5	[-24.0,19.0]	-2.6	[-20.9,25.8]
	STS-77*	10	6/8	-1.9	[-25.9,22.2]	*NA*	*NA*
	STS-78	17	6	-0.1	[-21.7, 21.5]	5.0	[-14.3,24.4]
Rats Overall				**-8.9**	**[-28.1,10.3]**	*NA*	*NA*
Rodents Overall				**-13.5**	**[-27.1,0.1]**	**-0.1**	**[-38.8,38.7]**
				I^2 = 51.9		**I^2 = 0**	

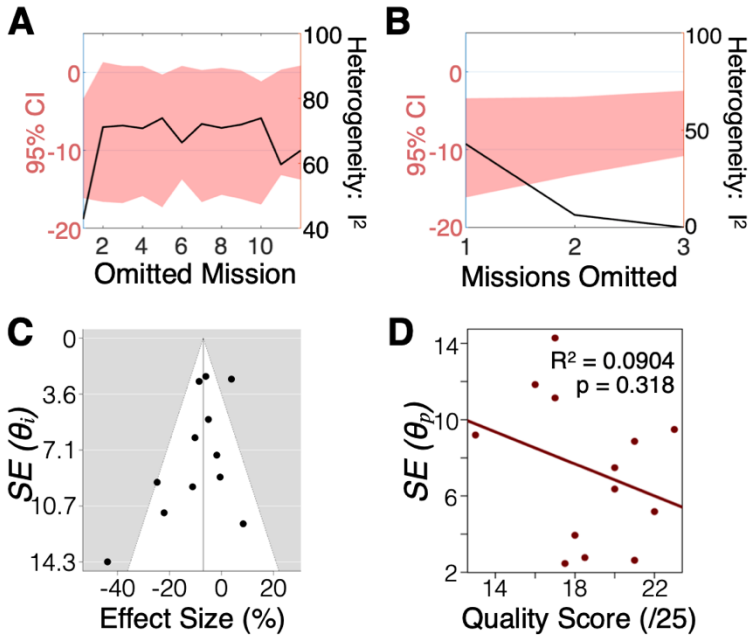
Days = mission duration; n_{SF} = spaceflight animal group sample size; n_{GC} = ground control sample size (only indicated if differ from SF group). SF vs GC indicates outcomes of spaceflight to ground control comparisons. GC vs VC indicates outcomes of ground control to vivarium control comparisons. ES (%) = effect size or percent difference; 95% CI = 95% confidence interval.

* = Mission outcomes where GC not present, and a VC is used as the comparison control

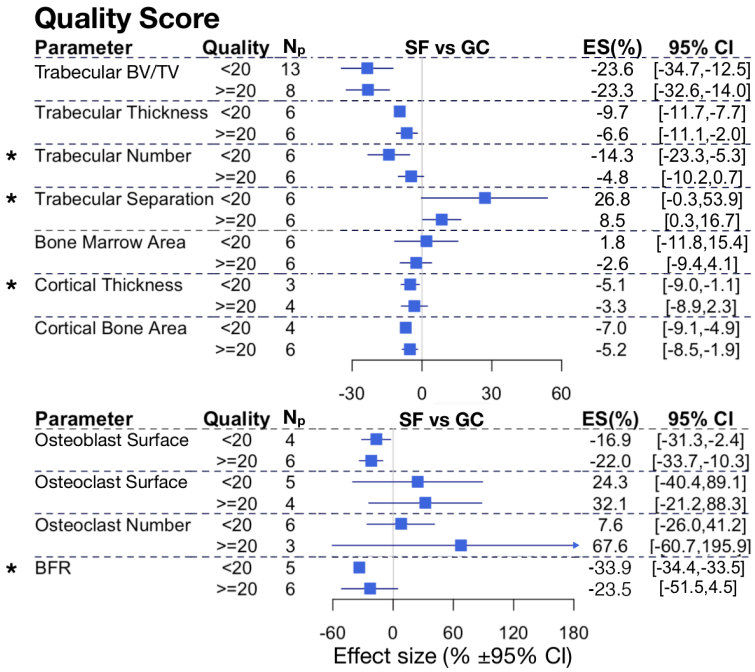
= contains measures of bone marrow area derived from marrow diameter

Supplementary Figures

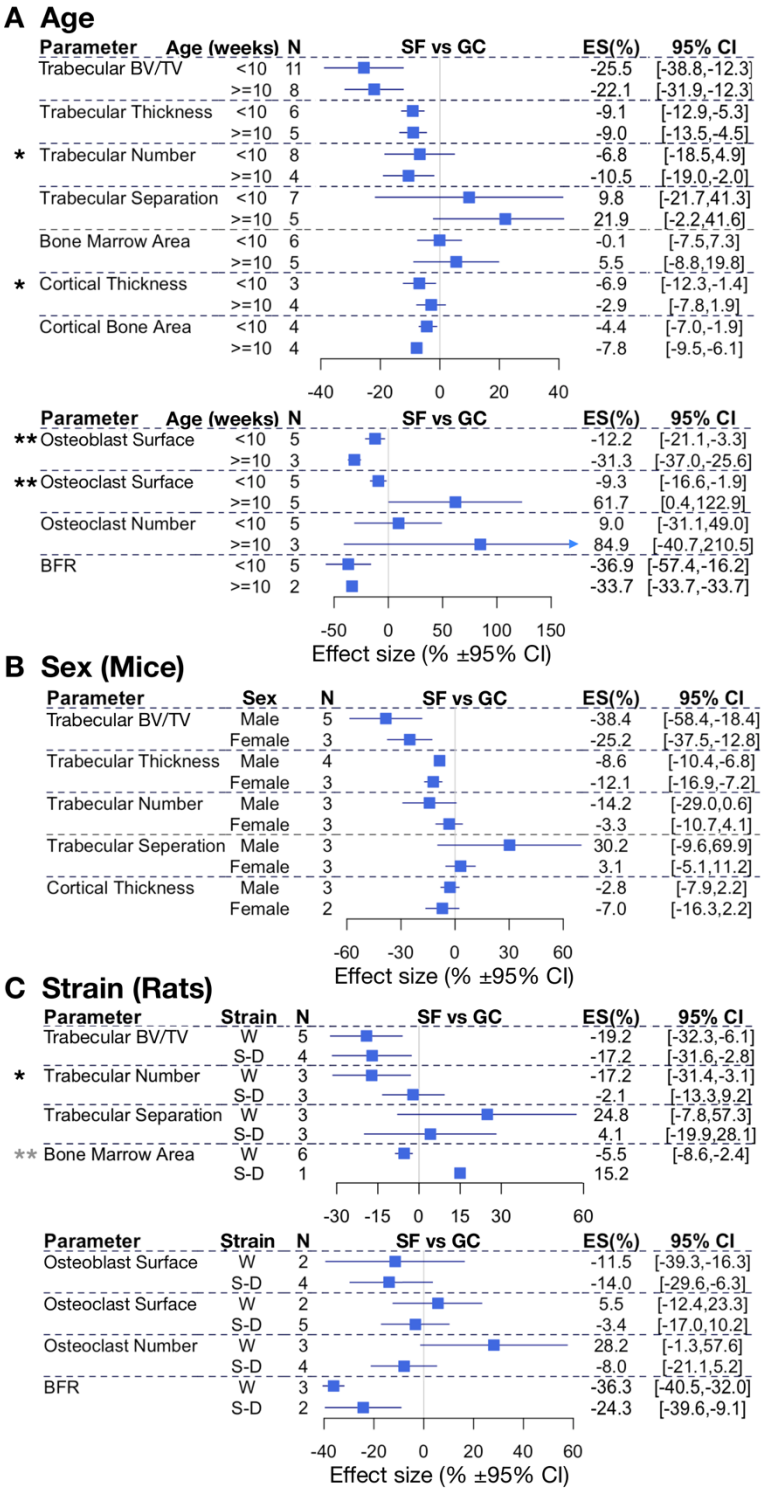
Supplementary Figure 1. Heterogeneity and sensitivity analyses for Tb.N. A, B) Heterogeneity was analyzed using single mission exclusion (A) and cumulative mission exclusion (B). Red area: 95% CI for the global effect size (left axis); line: I^2 (right axis). C) Funnel plot; D) article-level standard error $SE\ (\theta_P)$ as a function of quality score. R^2 and p-value is shown.



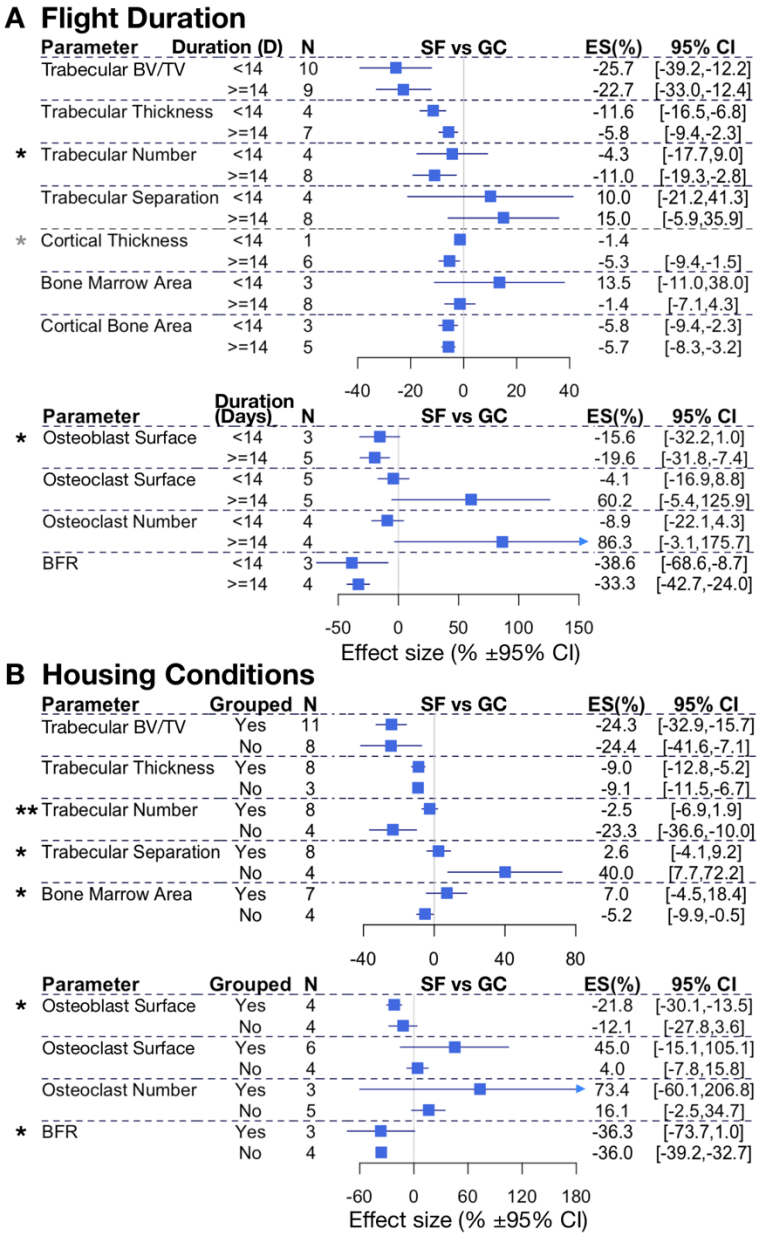
Supplementary Figure 2. Sub-group analysis of reported outcome by paper quality score. Rodent paper-level outcomes were divided into two groups, with quality score ≥ 20 or quality score < 20 on a 25-point scale. N_P is number of papers-level outcomes. Square/line: overall effect size (ES(%)) and 95% CI, numerical values of each are presented on the right. * indicate parameters for which the subgroups differ in their statistical significance from zero.

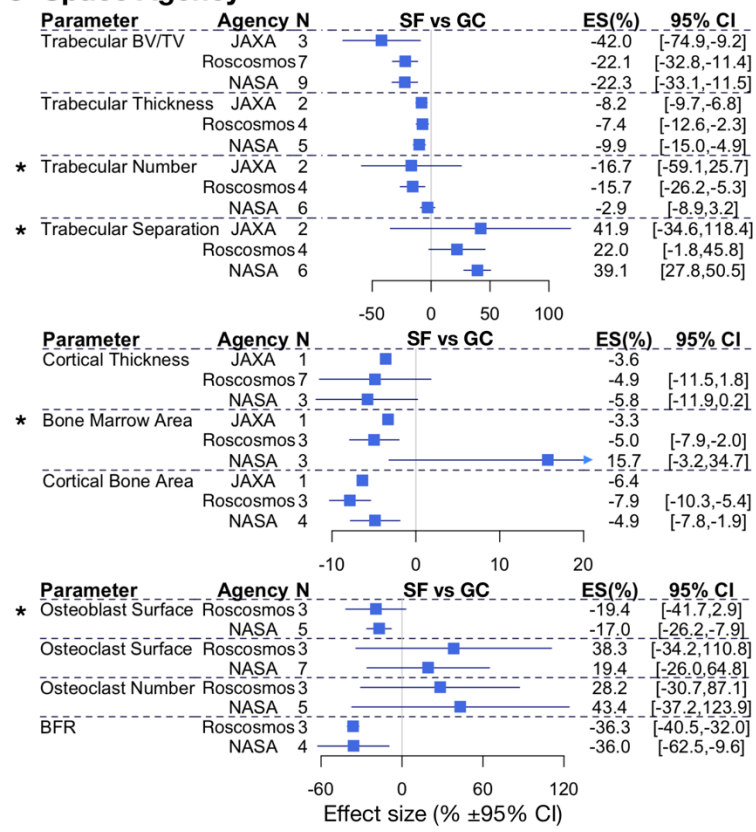


Supplementary Figure 3. Animal related subgroups. Rodent mission-level outcomes divided by animal age (A), sex (B), and strain (C). (A) Age at launch was <10 weeks (young animals) or ≥ 10 weeks (older animals). (B) Only mice missions were included, as all rat studies were performed exclusively with males. (C) Only rat missions were included for Wistar rats (W) and Sprague-Dawley rats (S-D), as all mice studies were performed with variants of the C57BL/6 lineage. N is number of mission-level outcomes in each sub-group. Square/line: overall effect size (ES(%)) and 95% CI, numerical values of each are presented on the right. * indicate parameters for which the subgroups differ in their statistical significance from zero. ** indicate parameters in which subgroups are significantly different from one another.

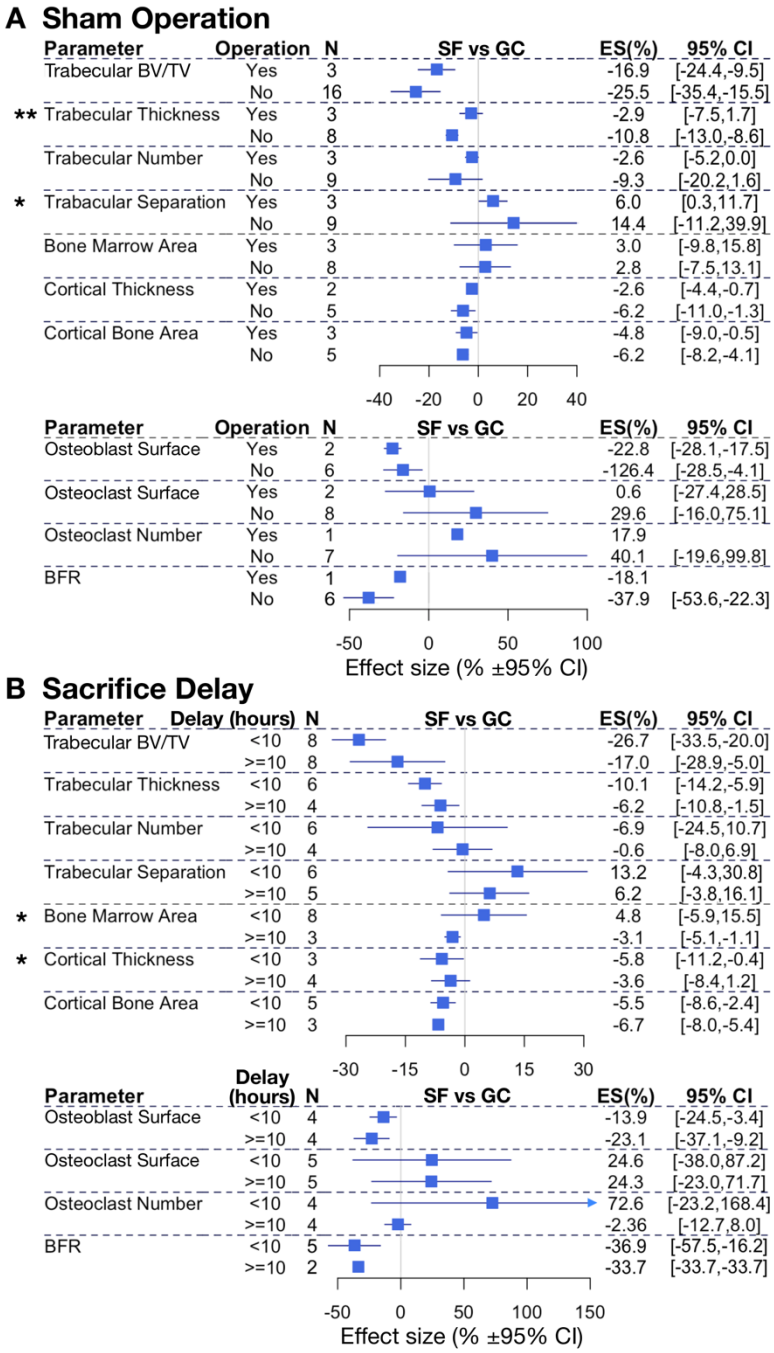


Supplementary Figure 4. Mission related subgroups. Rodent mission-level outcomes divided by mission-duration (A), single vs grouped housing condition (B), and space agency (C). (A) Mission duration subgroups: short duration missions (<14 days); long duration missions (>= 14 days). (B) Housing condition subgroups: animals housed as a group (Yes) and those individually housed (No). (C) Space agency subgroups: NASA, Roscosmos, and JAXA. N is number of mission-level outcomes in each sub-group. Square/line: overall effect size (ES(%)) and 95% CI, numerical values of each are presented on the right. * indicate parameters for which the subgroups differ in their statistical significance from zero. ** indicate parameters in which subgroups are significantly different from one another.





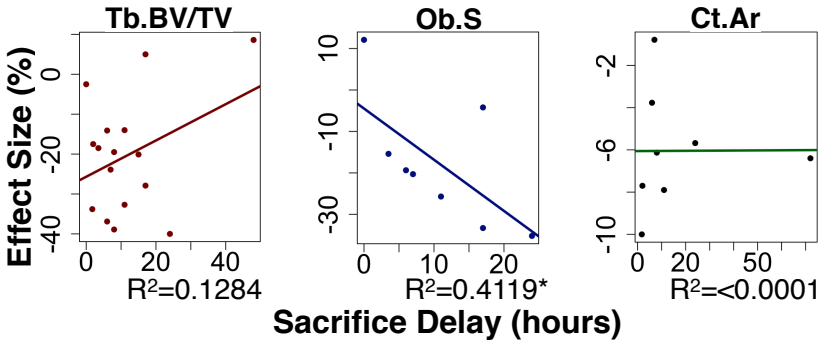
Supplementary Figure 5. Study related subgroups. Rodent mission-level outcomes divided by presence of sham operation (A) sacrifice delay (B), and ground control conditions (C). (A) Sham operation subgroups: missions that report performing sham operations (Yes); mission that report no sham operations (No). (B) Sacrifice subgroups: within 10 h of landing (<10) and longer than 10 h after landing (>=10). (C) specific conditions of ground control animals for each subgroup are indicated. N is number of mission-level outcomes in each sub-group. Square/line: overall effect size (ES(%)) and 95% CI, numerical values of each are presented on the right. * indicate parameters for which the subgroups differ in their statistical significance from zero. ** indicate parameters in which subgroups are significantly different from one another.



Ground Control Conditions

1. housed in same habitat as the spaceflight group, but not all conditions (e.g. food, and/or temperature, light/dark cycle, etc.) mimicked
2. housed in same habitat as the spaceflight group, and all conditions mimicked, not including force of liftoff or re-entry
3. housed in same habitat as the spaceflight group, and all conditions mimicked, including the force of lift-off and/or re-entry are simulated

Supplementary Figure 6. Meta-regression analysis of sacrifice delay. Mission-level outcomes, measured in effect size (%), of Tb.BV/TV, Ob.S, and Ct.Ar were plotted as a function of sacrifice delay of spaceflight animal group (hours). R^2 is shown. * indicated high R^2.



Supplementary Figure 7. Sub-group analysis of bone regions. Rodent bone measurement-level outcomes divided by region: region 1= skull, vertebra, and thorax; region 2 = pelvis, humerus, and femur; region 3 = tibia and ankle bones. N_j is number of bones measured in each sub-group. Square/line: overall effect size (ES(%)) and 95% CI, numerical values of each are presented on the right. * indicate parameters for which the subgroups differ in their statistical significance from zero. ** indicate parameters in which subgroups are significantly different from one another.