Effects of a Four-day Spaceflight and Recombinant Human Growth Hormone on Cancellous Bone Microarchitecture in Femoral Head of Rapidly Growing Male Rats

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Abstract

Spaceflight results in reduced bone accrual and muscle atrophy in growing rodents. Some studies suggest that the detrimental effects of spaceflight are due, in part, to impaired growth hormone (GH) signaling. An experiment flown aboard STS-41 (October 6–10, 1990) evaluated the efficacy of recombinant human growth hormone (rhGH) in ameliorating the detrimental effects of spaceflight on the musculoskeletal system in male Sprague Dawley rats. The rats were 39 days old at launch and sacrificed following the 4–day flight. Ground controls (n=11/treatment group) and flight animals (n=8/treatment group) were treated with rhGH or excipient delivered using osmotic pumps implanted subcutaneously one day prior to launch. For the present examination, cancellous bone in the femoral head was evaluated using X-ray microtomography (microcomputed tomography), a technology not available when the study was performed. Spaceflight resulted in lower cancellous bone volume fraction, connectivity density, trabecular thickness and trabecular number, and higher trabecular separation. rhGH had no independent effect on cancellous bone architecture and there were no spaceflight by rhGH interactions. These findings suggest that a short interval of microgravity during rapid growth was sufficient to reduce accrual of cancellous bone and alter bone microarchitecture at an important weight bearing skeletal site. Additionally, increasing growth hormone levels was ineffective in preventing cancellous osteopenia in flight animals and did not increase cancellous bone volume fraction in ground controls.

Introduction

Microgravity during spaceflight negatively influences the musculoskeletal system of rodents, but the precise effects on bone are affected by age, duration of flight, housing conditions (individual versus group housed), and skeletal site (weight bearing versus non-weight bearing) [1] [2] [3]. Weight bearing skeletal sites are generally more sensitive to microgravity than non-weight bearing sites, supporting an important physiological role for impact loads generated during dynamic weight bearing [4].

Most spaceflight studies investigating the musculoskeletal system in growing rodents pre-date widespread availability of three-dimensional high-resolution nondestructive imaging. Consequently, the effects of microgravity on bone microarchitecture are not well defined. This paucity in information constrains our ability to model the association between local changes in skeletal loading status during spaceflight and resulting alterations in architecture responsible for degradation of bone mechanical properties [5] [6]. Such knowledge is essential to design and evaluate effective interventions that minimize detrimental skeletal changes during long duration exposure to microgravity. In order to address the gap in knowledge, the present study evaluated the femoral head, a clinically relevant but rarely evaluated skeletal site in animal models. The rats were flown in 1990 on the Space Shuttle Discovery (STS-41) and bones archived following recovery. Based on evidence that reduced bone accrual and muscle atrophy in growing rodents during spaceflight are due, in part, to impaired growth hormone (GH) signaling [5] [6], the study design included ground controls and flight animals treated with recombinant human GH (rhGH). The original goal was to establish the efficacy of this intervention in attenuating the detrimental effects of spaceflight on the musculoskeletal system.

Objective

The objectives of the present analysis are to determine if a 4–day spaceflight alters cancellous bone microarchitecture in the femoral head of rapidly growing male Sprague Dawley rats and to assess whether rhGH has a positive effect on cancellous bone parameters.

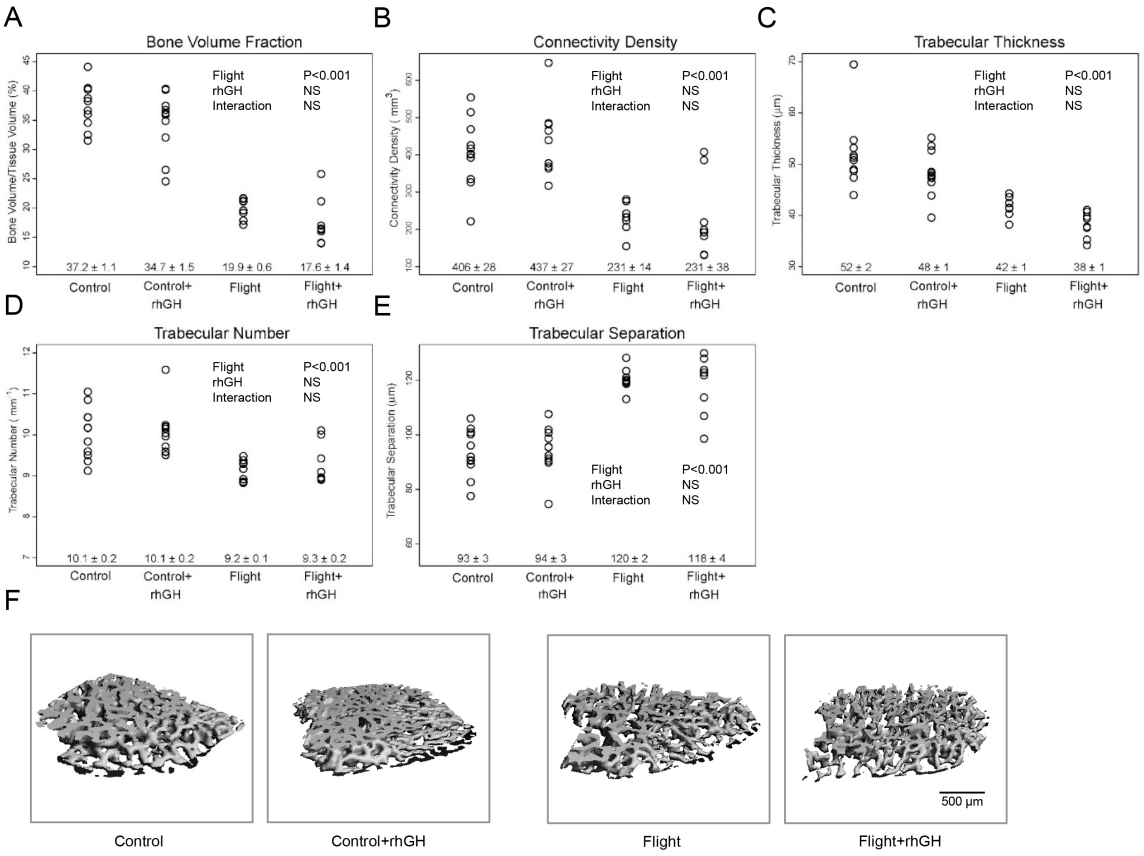

Figure 1

a

Figure Legend

Figure 1. The effects of a 4–day spaceflight on cancellous bone microarchitecture in femoral head of growing male Sprague Dawley rats.

The flight resulted in lower **(A)** cancellous bone volume fraction, **(B)** connectivity density, **(C)** trabecular thickness, and **(D)** trabecular number, and higher **(E)** trabecular separation. Representative three-dimensional images of cancellous bone from animals in each treatment group are shown in panel **(F)**.

Data were analyzed using two-way ANOVA and are reported as dot plots and mean + SEM. n=11/group (control groups) and 8/group (flight groups).

Results & Discussion

The effects of spaceflight and rhGH treatment on cancellous bone microarchitecture in the femoral head are shown in figure 1. The 4–day flight resulted in lower cancellous bone volume fraction (A), connectivity density (B), trabecular thickness (C) and trabecular number (D), and higher trabecular separation (E). Treatment with rhGH had no

significant effect on the endpoints measured. Significant spaceflight by rhGH interactions were likewise not detected for any of the endpoints evaluated. Alterations in the bone microarchitecture in flight animals can be readily appreciated in three-dimensional images of the cancellous bone compartment evaluated in the femoral head (F).

Histomorphometric analysis of muscle and bone in male Sprague Dawley rats flown on STS-41 detected soleus atrophy but no change in femur length, cortical bone cross-sectional area in femur diaphysis or cancellous bone volume fraction in the distal femur metaphysis [7] [8]. rhGH had no effect on soleus muscle but stimulated periosteal bone formation in the femur diaphysis in control and flight animals. The inability to detect changes in bone microarchitecture following this 4–day flight by conventional histomorphometry may have been due to limitations of two-dimensional analysis. Alternatively, the femoral head evaluated in the present study may be more sensitive to skeletal unloading. A 14–day spaceflight on STS-58 resulted in region-specific changes in gene expression levels for bone matrix proteins [9]. Notably, the proximal femur from flight animals on STS-58 exhibited lower mRNA levels for osteocalcin, osteonectin and type 1 collagen. The distal femur metaphysis from flight animals exhibited reduced mRNA levels for osteonectin and type 1 collagen but not osteocalcin and no change in mRNA levels occurred in midshaft diaphysis or distal femur epiphysis. These findings provide support for the possibility that the proximal femur is highly sensitive to microgravity.

To our knowledge, the effect of spaceflight on the femoral head has not been previously evaluated. STS-62 (14–day spaceflight using ovariectomized Fisher 322 rats) is the only spaceflight study reporting three-dimensional microarchitecture of rat bone. Cancellous bone loss occurred in flight animals in lumbar vertebra and distal femur metaphysis but not in distal humerus [10]. Bone in the proximal femur was not evaluated.

Because of the very young age of the rats in the present study, it is likely that the lower bone volume in the femoral head of flight animals reflects reduced bone accrual during growth rather than net bone loss; lower trabecular thickness and lower trabecular number contributed to the lower cancellous bone volume fraction. The change in microarchitecture is consistent with increased resorption of primary spongiosa (reduced trabecular number) in combination with reduced addition of bone onto primary spongiosa (reduced trabecular thickness). This proposed cellular mechanism is consistent with results of studies evaluating the response of cancellous bone to spaceflight in tibia and femur by dynamic histomorphometry [11] [12] [13].

Cancellous bone loss occurred in the distal femur metaphysis and epiphysis of ovariectomized Fisher 322 rats flown on STS-62 (14–day spaceflight) [10]. It is noteworthy that the most severe bone loss during long duration spaceflight in astronauts is in the proximal femur [14] [15]. Thus, future studies performed in more skeletally mature animals should evaluate this site thoroughly.

GH plays an important role in bone growth and maturation and reduced GH signaling has been proposed to contribute to bone loss during spaceflight [16] [17] [18] [19], in part, due to a microgravity-induced secretory dysfunction [20]. GH has potent anabolic actions on the musculoskeletal system and there has been long-term interest in whether GH, alone or in combination with other anabolic agents, has therapeutic value in prevention of disuse osteopenia and sarcopenia [21]. In the present study, treatment with rhGH did not prevent the skeletal changes incurred during spaceflight. This finding does not support the hypothesis that decreased GH secretion, which when severe is known to reduce bone formation in growing rats, is responsible for reduced bone accrual during spaceflight [22] [23]. However, the study does not rule out the possibility that increasing GH levels above normal may partially counteract the detrimental skeletal effects of microgravity. Here we focused on cancellous bone in a region where there was no response to increased levels of the hormone but some research suggests that GH has a more potent effect on cortical bone [24].

Microgravity experiments in mice are being performed using a variety of platforms, including the International Space Station, but to date studies detailing bone microarchitecture have been few in number [5] [25] [26]. In the future, it should be possible

to establish whether there is concordance in microgravity-associated changes in bone microarchitecture between rats and mice. Similar responses in both species would add credence to the generalizability of the current results. Additional analysis of archived tissue from space shuttle studies using rats would contribute to achieving this goal.

Conclusions

Dynamic weight bearing is important for optimal bone accrual during growth. Our analysis illustrates that duration of skeletal unloading required to reduce bone accrual and alter bone microarchitecture during microgravity is surprisingly short. We also show that treatment with rhGH is ineffective in preventing the detrimental effects of spaceflight on cancellous bone mass and microarchitecture in the femoral head of rapidly growing male rats.

Limitations

The altered microarchitecture in flight rats would likely result in reduced bone strength, which is consistent with reduction in femur strength reported in astronauts [14]. Long-term storage of bone in alcohol (as done in the current study) is not ideal for destructive mechanical testing, but in the future it may be possible to estimate the magnitude of change in mechanical properties using finite element modeling [27].

The duration of the spaceflight mission was only 4 days and the animals were growing rapidly. Longer duration spaceflight studies in growing and skeletally-mature animals are recommended to determine the effects of spaceflight on the adult skeleton.

Alternative Explanations

Spaceflight results in a transient reduction in the rate of weight gain. Thus, it is possible that reduced weight contributes to the decrease in bone accrual during short duration spaceflight. This possibility can be tested using a model for simulated microgravity (hindlimb unloading) and pair-feeding normal weight-bearing controls to unloaded animals to equalize weight gain.

Conjectures

Spaceflight opportunities will continue to be rare, emphasizing the wisdom of archiving tissues for future analysis, preferably using non-destructive approaches. Archived bone specimens from longer duration spaceflight studies should be analyzed to determine whether the changes in bone mass and architecture observed in the head of the femur persist. Cortical bone microarchitecture should also be evaluated to determine whether it is influenced by spaceflight and/or rhGH.

Additional Information

Methods

The details of this spaceflight experiment aboard STS-41 (Physiological Systems Experiment 1), including delivery of rhGH, have been described [28]. Briefly, the experiment included four groups of rats, ground control (n=11), ground control treated with rhGH (n=11), flight (n=8) and flight treated with rhGH (n=8). The rhGH-treated rats received a dose of 556 mg/d, achieving mean rhGH serum levels at sacrifice of 39.3 ng/ml in ground and 36.1 ng/ml in flight rats. The animals were 39 days old at launch and flown in space for 4 days.

For the current analysis, X-ray microtomography was used for three-dimensional evaluation of cancellous bone microarchitecture in femoral head. Right proximal femora

were scanned at a voxel size of 12 μm^3, 55 kVp X-ray voltage, 145 µA intensity, and 200 ms integration time using a Scanco µCT40 scanner (Scanco Medical AG, Basserdorf, Switzerland). Filtering parameters sigma and support were set to 0.8 and 1, respectively. The threshold for evaluation was set at 245 (gray scale, 0-1000). Twenty slices (240 µm in length) of cancellous bone were assessed in the proximal half of the femoral head. Measurements included cancellous bone volume fraction (bone volume/tissue volume, %), connectivity density (number of redundant connections per unit volume, mm^3; this index detects defects in cancellous architecture), trabecular thickness (mean thickness of individual trabeculae, µm), trabecular number (number of trabecular intercepts per unit length, mm^{-1}) and trabecular separation (distance between trabeculae, µm).

Statistics

Experimental data generated according to a 2×2 factorial design with categorical variables for spaceflight group (spaceflight and ground control) and treatment group (rhGH and vehicle control) were analyzed using a linear two-way analysis of variance model with an interaction between spaceflight and treatment group. Model assumptions were assessed using graphical techniques and statistical significance tests. Specifically, residual plots and Levene's test assessed homogeneity of variance, while quantile-quantile plots and Anderson-Darling tests assessed normality. To adjust for multiple comparisons, the maximum false discovery rate was set equal to 5% [29]. Differences were considered significant at $p \leq 0.05$. Data analysis was performed using R version 3.4.3.

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Ethics Statement

Archived tissue were obtained from the Center for Cell Research (Pennsylvania State University). The animal protocol was approved by the Animal Care and Use Committees at NASA and Genentech.

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