Lack of effect of spaceflight on bone mass and bone formation in group-housed rats

T. J. Wronski, M. Li, Y. Shen, S. C. Miller, B. M. Bowman, P. Kostenkuik, and B. P. Halloran. Lack of effect of spaceflight on bone mass and bone formation in group-housed rats. J. Appl. Physiol. 85(1): 279–285, 1998.—As part of an experiment to study the role of corticosteroids in bone changes during spaceflight, male Sprague-Dawley rats (6 wk old, 165 g body weight) were placed in orbit for 17 days, in groups of six, in animal-enclosure modules (AEMs) aboard the space shuttle Columbia (STS-78). Control rats were group housed in a similar manner in ground-based AEMs or standard vivarium cages. Adrenal hypertrophy occurred in flight rats, but bone histomorphometric analyses revealed a lack of significant changes in bone mass and bone formation in these animals. Cancellous bone volume and osteoblast surface in the proximal tibial metaphysis were nearly the same in flight and ground-based rats. Normal levels of cancellous bone mass and bone formation were also detected in the lumbar vertebrae and femoral necks of flight rats. In the tibial diaphysis, periosteal bone formation rate was found to be identical in flight and ground-based rats. The results indicate that, under conditions of group housing in AEMs, spaceflight has minimal effects on bone mass and bone formation in rapidly growing rats. These findings emphasize the need to investigate the importance of rat age, strain, and especially housing conditions for studies of the skeletal effects of spaceflight.

BONE LOSS is a potentially serious consequence of long-term spaceflight. Gemini, Apollo, and Skylab astronauts consistently exhibited hypercalcemia and negative calcium balances consistent with a loss of skeletal mass (12, 17). Photon absorptiometry confirmed that the bone mineral density of the calcaneus declined by ~4% in Skylab crewmembers after nearly 3 mo of orbital flight (16). More recently, Collet et al. (6) used peripheral quantitative computed tomography and ultrasound to measure bone mass in a cosmonaut before and after 6 mo of weightlessness aboard the Mir space station. They found a marked loss of bone mass in the calcaneus (−13.2%) and tibia (−4.5%) of the cosmonaut at the end of the flight period. Furthermore, the osteopenic tibia had not recovered completely by 6 mo after the return of the cosmonaut to Earth’s gravity. These findings are a cause for concern in view of plans for long-term human occupancy of the US space station and a possible extended mission to Mars.

The rat is the most commonly used animal model for detailed, invasive studies of bone changes during spaceflight. The early studies were confined to cortical bone. Morey and Baylink (14) determined that an inhibition of periosteal bone formation occurred in the tibial diaphysis of flight rats. The increased extent of arrest lines in these animals suggested that periosteal bone formation may even have ceased during spaceflight. These findings were confirmed in subsequent flight experiments (19, 24–26).Although cortical bone mass was maintained at normal levels in rats subjected to spaceflight, osteopenia was found to develop in the more metabolically active cancellous bone of these animals (9, 23, 27). The observed loss of cancellous bone in flight rats has been attributed primarily to decreased bone formation (9, 20, 23, 27). On the basis of histomorphometric and calcium kinetic analyses, most investigators have reported bone resorption to be normal or possibly decreased in rats during weightlessness (4, 9, 20, 23, 27). These findings are consistent with recent biochemical evaluations of bone turnover in cosmonauts (6).

Although it is commonly assumed that weightlessness induces pronounced bone changes in rats, the literature includes some hints of equivocal results. For example, Vico et al. (22) detected only a nonsignificant trend for cancellous bone loss in the proximal tibia of rats placed in orbit for 14 days. Bone biomechanical properties were also found to be normal in the humeri of these same flight rats (21). In the present study, we found that indexes of cancellous bone mass and bone formation were nearly identical in flight and ground-based control rats at the end of a 17-day flight experiment. These findings have important implications for the design of future studies utilizing the rat as an animal model for the adverse skeletal effects of spaceflight.

MATERIALS AND METHODS

Male Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY). They were ~45 days of age and weighed an average of 165 g at the time of launch. All procedures involving use of these rats were approved by the Institutional Animal Care and Use Committees at the University of Florida (Gainesville, FL) and at National Aeronautics and Space Administration (NASA)-Ames Research Center (Moffett Field, CA).
The study was originally designed to evaluate the potential role of corticosteroids in bone changes during spaceflight. One group of flight rats with intact adrenal glands was expected to experience adrenal hypertrophy and the bone changes associated with spaceflight. In a second group of flight rats, serum corticosteroids were to be maintained at physiological levels by a combination of adrenalectomy (ADX) and supplementation with corticosteroids via implanted hormone pellets. By comparisons with appropriate ground-based control rats, it would be determined whether the maintenance of serum corticosteroids at normal levels in flight rats would affect the incidence of bone changes during spaceflight.

At 4 days before launch, all rats were anesthetized with ketamine and xylazine at doses of 50 and 10 mg/kg body weight, respectively, and subjected to sham surgery or bilateral ADX. Pellets composed of corticosterone (30%) and aldosterone (0.15%) dissolved in cholesterol (~100 mg) were implanted in the dorsal scapular region of each ADX rat. Placebo pellets composed of cholesterol alone were implanted in sham-operated rats. The components of the pellets were obtained from Steroloids (Wilton, NH). The corticosteroid pellets were designed to deliver physiological levels of corticosterone and aldosterone to the systemic circulation of ADX rats.

On the day before launch, six sham-operated rats and six ADX rats were injected with calcine (15 mg/kg body weight sc) to label bone-forming surfaces. The rats were then placed into two animal-endosure modules (AEM) (6 rats/AEM). These AEMs were loaded soon afterwards into mid-deck stowage lockers of the space shuttle Columbia, which was launched the following day for a 17-day mission (STS-78). The temperature within the AEMs varied between 22 and 28°C during the mission. The flight rats were exposed to 12 consecutive hours of light during each 24-h period.

Six baseline sham-operated rats and six baseline ADX rats were killed on the day of launch. On the day after launch (48 h after calcine labeling of flight rats), all ground-based rats were injected with calcine as described above. Soon afterwards, six sham-operated rats and six ADX rats were placed in two ground-based AEMs (6 rats/AEM), while an additional six sham-operated rats and six ADX rats were placed in standard vivarium cages (6 rats/cage). Therefore, the experiment consisted of the following eight groups of rats (n = 6 per group): 1) baseline sham, 2) baseline ADX, 3) flight sham, 4) flight ADX, 5) AEM sham, 6) AEM ADX, 7) vivarium sham, and 8) vivarium ADX.

During the flight period, the ground-based rats of groups 5–8 were housed in the animal resources facility at Kennedy Space Center, FL. The AEMs and vivarium rooms were maintained at a temperature of 28°C with a 12:12-h light/dark cycle. Food and water were available ad libitum to all flight and ground-based rats. The food consisted of hydrated bars (Teklad, Madison, WI) with calcium and PO4 contents of 0.63 and 0.49%, respectively.

The sham and ADX flight rats were killed by decapitation 4–7 h after landing from a 17-day spaceflight. Ground-based sham and ADX rats housed in AEMs and vivarium cages were killed in the same manner 48 h later. Blood was collected from each rat at necropsy and stored as serum at −80°C. The adrenal glands from the sham rats were dissected free from surrounding tissues and were weighed immediately with a Mettler balance. The right proximal and distal halves of the tibia, right proximal femur, and first lumbar vertebra were stripped of musculature and placed in 10% phosphate-buffered Formalin for 24 h for tissue fixation. The bone samples were then stored in 70% ethanol before histological processing.

The corticosterone and aldosterone concentrations were measured in serum samples by using radioimmunoassay methods with a commercially available kit (Diagnostic Products, Los Angeles, CA).

Bone samples other than the distal one-half of the tibia were dehydrated in ethanol and embedded undecalcified in methyl methacrylate (2). Longitudinal sections were cut with an Isomet low-speed saw (Buehler, Lake Bluff, IL) and viewed with polarized light microscopy. The bone samples were stained with silver nitrate alone and subjected to trabecular structural analyses, as described previously (13). Briefly, an image-analysis system (KSS Scientific Consultants, Magna, UT) was used to define cancellous (trabecular) bone patterns, or strut types, within the proximal tibial metaphysis as free to end-to-end (free-free), node to free end (node-free), or node to node (node-node). The percentage of each strut type was calculated with software obtained from KSS Scientific Consultants. These data are indexes of trabecular connectivity and cancellous bone architecture. For example, the percentage of free-free struts increases, whereas the percentage of node-node struts decreases at osteopenic skeletal sites in rats; this indicates a loss of trabecular connectivity (13).

The distal one-half of the right tibia from each rat was dehydrated in 100% ethanol and acetone and was then embedded undecalcified in a styrene monomer that polymerizes into a polyester resin (Tap Plastics, San Jose, CA). The tibial diaphysis 1- to 2-mm proximal to the tibiofibular junction was sawed into 75-µm-thick cross sections with an Isomet low-speed saw. The cross sections were stained with silver nitrate and subjected to biochemical analyses, as described previously (13). Briefly, the sample areas were divided by the same time interval to calculate perosteal and endocortical bone formation rates in units of micrometers/day. Similarly, the area of newly formed cortical bone between the fluorescent calcine label and the perioseal surface was measured at a magnification of ×200, as well as that portion of the perioseal surface covered by osteoblasts alone and of osteoblasts together with osteoclasts, as percentages of total cancellous tissue area. In addition, cortical width was measured from the perioseal to the endocortical surfaces at four equally spaced sites at the anterior (cranial), posterior (caudal), medial, and lateral aspects of each cross section. The four measurements were averaged to obtain a mean cortical width for each animal. Under ultraviolet illumination, the area of newly formed bone between the fluorescent calcine label and the perioseal surface was measured at a magnification of ×100. Also, the distance between the calcine label and the perioseal surface was measured at 100-µm intervals around the periphery of cortical bone at a magnification of ×200. This distance was divided by the time interval between administration of the calcine label and the time when the animal was killed (18 days) to calculate perioseal mineral apposition rate in units of micrometers/day. Similarly, the area of newly formed cortical bone between the calcine label and the perioseal surface was divided by the same time interval to calculate the perioseal bone formation rate in units of millimeters/day. All data are expressed as the means ± SD for each group.
by ANOVA followed by Fisher’s protected least significant difference test for multiple comparisons. P values < 0.05 were considered to be significant.

RESULTS

All rats gained substantial body weight during the course of the experiment (Table 1). The sham and ADX flight rats exhibited at least as much weight gain as the sham and ADX ground-based rats. In fact, the mean body weight of the ADX flight group was slightly but significantly increased compared with the mean body weight of all other groups.

The mean adrenal gland weight for the flight sham group was significantly greater than that of the other three sham groups (Table 1). The ground-based AEM and vivarium sham rats exhibited a nonsignificant trend for increased adrenal gland weight compared with that of the younger baseline sham rats.

Mean values for serum corticosterone and aldosterone are listed in Table 1. Flight sham rats with intact adrenal glands had a significantly higher mean value for serum corticosterone (~500 ng/ml) by at least a factor of two compared with all other groups. The ground-based AEM and vivarium sham groups also exhibited increased serum corticosterone compared with their respective ADX + hormone-replacement groups. In contrast, the mean value for serum corticosterone remained at a physiological level of 50–60 ng/ml in all ADX + hormone-replacement groups. Similarly, mean serum aldosterone (Table 1) was maintained at a physiological level of 80–100 pg/ml in the three ADX + hormone-replacement groups.

No substantial differences in bone histomorphometric variables were detected between the sham rats with intact adrenal glands and the ADX rats supplemented with physiological levels of aldosterone and corticosterone. Therefore, bone data from only the sham groups are presented.

Mean values for cancellous bone volume in the proximal tibial metaphysis and lumbar vertebral body are shown in Fig. 1, A and B, respectively. This index of cancellous bone mass was nearly the same in the flight sham group and both groups of ground-based sham rats, with no significant differences among the groups at either skeletal site. Similarly, flight sham rats did not exhibit decreased cancellous bone volume in the femoral neck (data not shown), as the mean value for this group (34.5%) was not significantly different from the means for the AEM sham (37.4%) and vivarium sham (33.7%) groups.

Indexes of trabecular connectivity in the proximal tibia of flight sham rats were also not significantly different from those of the AEM sham and vivarium sham groups (data not shown). For example, mean values for free-free struts, which are known to increase in osteopenic states (13), were 20.6, 24.0, and 24.0% for the above three groups, respectively.

Osteoclast surface, an index of bone resorption, was significantly decreased in the proximal tibia of the flight sham rats compared with the baseline sham and AEM sham groups (Fig. 1). Mean values for osteoclast surface in the lumbar vertebrae varied little among groups (Fig. 1D).

Mean values for osteoblast surface, an index of bone formation, in the proximal tibial metaphysis and lumbar vertebral body are shown in Fig. 1, E and F, respectively. No significant differences in osteoblast surface at either skeletal site were detected among the four groups of sham rats.

Histomorphometric data from cortical bone in the tibial diaphysis are shown in Fig. 2. An age-related increase in cortical bone area (Fig. 2A) and width (Fig. 2B) occurred in the flight, AEM, and vivarium sham groups compared with the baseline sham group, but no significant differences were observed among the former three groups. Mean marrow area was nearly the same (1.1 mm²) in all four groups of sham rats. Age-related decreases in peristomal bone formation rate (Fig. 2C) and mineral apposition rate (Fig. 2D) were detected in the older flight, AEM, and vivarium sham groups compared with the younger baseline sham group. However, the mean values for these variables were very similar in the former three groups, with no significant differences among them.

DISCUSSION

This study was designed to determine whether corticosteroids contribute, at least in part, to bone changes during spaceflight. Based on past reports (8, 9, 14, 15, 20, 23–27), the flight rats with intact adrenal glands were expected to develop adrenal hypertrophy and skeletal abnormalities in response to spaceflight. Adrenal hypertrophy was observed in these animals, which suggests that corticosteroid excess occurred as a stress response to the weightless environment. To evaluate the skeletal consequences of the observed corticosteroid excess in flight rats, it was essential to maintain serum corticosteroids at a physiological level in a second group of flight rats by a combination of ADX (to eliminate endogenous corticosteroids) and corticosteroid supplementation via implanted hormone pellets. This effort was successful, because the serum concentra-

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**Table 1. Body and gland weights and serum concentrations of corticosteroids**

<table>
<thead>
<tr>
<th>Group</th>
<th>Final Body Weight, g</th>
<th>Adrenal Gland Weight, mg</th>
<th>Serum Corticosterone, ng/ml</th>
<th>Serum Aldosterone, pg/ml</th>
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<tr>
<td>Baseline</td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>174 ± 14</td>
<td>29.5 ± 3.7</td>
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<tr>
<td>ADX</td>
<td>177 ± 14</td>
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<tr>
<td>Flight</td>
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<tr>
<td>Sham</td>
<td>281 ± 22</td>
<td>40.5 ± 3.9*</td>
<td>498 ± 77*</td>
<td>438 ± 142*</td>
</tr>
<tr>
<td>ADX</td>
<td>309 ± 8*</td>
<td></td>
<td>65 ± 24*</td>
<td>104 ± 54*</td>
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<tr>
<td>AEM</td>
<td></td>
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<tr>
<td>Sham</td>
<td>270 ± 13</td>
<td>33.8 ± 2.2</td>
<td>181 ± 117</td>
<td>427 ± 230</td>
</tr>
<tr>
<td>ADX</td>
<td>273 ± 18</td>
<td></td>
<td>52 ± 17*</td>
<td>78 ± 19*</td>
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<tr>
<td>Vivarium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>262 ± 12</td>
<td>33.9 ± 7.2</td>
<td>165 ± 172</td>
<td>370 ± 219</td>
</tr>
<tr>
<td>ADX</td>
<td>265 ± 11</td>
<td></td>
<td>60 ± 21*</td>
<td>92 ± 31*</td>
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</table>

Values are means ± SD. ADX, adrenalectomized; AEM, animal enclosure modules. All ADX rats were supplemented with corticosterone and aldosterone. * Significantly different from all other groups (P < 0.05). † Significantly different from sham groups (P < 0.05).
tions of corticosterone and aldosterone in the ADX + hormone-replacement rats were equivalent to the normal 24-h mean for these hormones (7). Despite the successful implementation of this aspect of the study, the experimental goals could not be achieved because of the failure of bone changes to develop in flight rats with intact adrenal glands. This finding is somewhat surprising, because previous investigators have reported loss of cancellous bone mass (9, 23, 27) and an inhibition of periosteal bone formation (14, 19, 24–26) in rats subjected to spaceflight. In contrast, the flight rats of the present study failed to exhibit even a trend for cancel-
Lack of bone changes in rats during spaceflight.

Fig. 2. Values (means ± SD) in tibial diaphysis for cortical bone area (A), cortical width (B), periosteal bone formation rate (C), and periosteal mineral apposition rate (D). See Fig. 1 legend for details. *Significantly different from baseline sham rats, P < 0.05.

Lack of bone changes or depressed periosteal bone formation. Trabecular connectivity was also found to be normal in the proximal tibia of flight rats.

The reasons for the conflicting results are unclear. Possible explanations include differences in duration of spaceflight, housing conditions aboard the spacecraft, and age and strain of rats. The first possibility, duration of spaceflight, does not seem to be an important factor in the present study, because the 17-day flight period should have been adequate in duration for bone changes to develop. In support of this contention, rats placed in orbit for 18–20 days aboard Soviet Cosmos biosatellites exhibited marked loss of cancellous bone and a strong inhibition of bone formation (9, 14, 19, 25). Furthermore, similar bone changes have been reported to occur in rats after only 7–14 days of weightlessness (20, 23, 27).

To emphasize the importance of housing conditions during spaceflight, perhaps the most revealing studies are those of Vico et al. (22, 23). Both of those studies involved use of male Wistar rats that were of an identical age (105 days) at launch. In the earlier study (23), the rats were placed in orbit for 7 days in a Cosmos biosatellite while singly housed. In the later Cosmos study (22), the rats were placed in orbit for 14 days while group housed. Despite the shorter duration of spaceflight in the earlier study, the singly housed flight rats exhibited a highly significant (47%) loss of cancellous bone in the proximal tibia and a 56% decrease in osteoid surface, an index of bone formation. In contrast, the group-housed flight rats of the later, longer study had a nonsignificant 18% decrease in cancellous bone mass as well as a nonsignificant 22% decrease in osteoid surface at the same skeletal site. Perhaps the greater activity levels associated with the social interactions of group housing minimize the skeletal effects of spaceflight in rats. This speculation is unsupported by data at present and requires confirmation. On the other hand, several investigators have detected bone changes in group-housed flight rats, including decreased gene expression for bone matrix proteins (1, 3) and an inhibition of periosteal and cancellous bone formation (1, 20, 24). Those results, which conflict with the negative findings of the present study and previous studies (21, 22), emphasize the need for definitive investigations of the importance of rat housing conditions for the development of the adverse skeletal effects of spaceflight.

Age is an important consideration in all rat studies, because rapid bone growth in young rats complicates extrapolation of findings from these animals to adult...
human bone. Furthermore, bone changes in response to an experimental manipulation may take longer to develop in slowly growing, aged rats relative to rapidly growing, young rats. Nevertheless, past and present findings suggest that the magnitude of the skeletal response to spaceflight is more dependent on housing conditions than on age. For example, singly housed flight rats exhibited significant bone changes regardless of whether their age at launch was 55–105 days (9, 14, 23, 25, 27). Conversely, group-housed flight rats failed to exhibit substantial bone changes regardless of whether they were 45 days of age, as in the present study, or 105 days of age at launch (21, 22).

At first glance, strain of rats also does not appear to influence bone changes during spaceflight to the same extent as housing conditions. Fischer 344, Sprague-Dawley, and Wistar rats have all exhibited a strong skeletal response to spaceflight (5, 14, 20, 23–25, 27). However, in contrast to Sprague-Dawley rats in the present study and Wistar rats (22), Westerlind and Turner (24) reported a highly significant 35% decrease in bone formation in group-housed Fischer 344 rats placed in orbit for 11 days. Although the results of a single study are not definitive, their finding suggests that the skeletal effects of weightlessness under conditions of group housing may be more pronounced in rats of the latter strain.

The observation of adrenal hypertrophy in flight rats is suggestive of corticosteroid excess in these animals in response to the stressful environment of space. Other investigators have also detected adrenal hyper trophy in rats subjected to spaceflight (8, 15). In the present study, the flight rats with intact adrenal glands also exhibited markedly increased terminal levels of serum corticosterone that were approximately an order of magnitude greater than the normal 24-h mean for the hormone. However, this gross corticosteroid excess at the conclusion of the experiment is probably due to the short-term stress of reentry and postflight handling rather than being reflective of a sustained, large increase in serum corticosterone throughout the 17-day duration of the mission. Nevertheless, the observation of adrenal hypertrophy in flight rats, which probably developed over the relatively long flight period, suggests that serum corticosterone was elevated in these animals during spaceflight. Even a moderate increase in serum levels of this hormone for 3 wk has adverse skeletal effects in rats. Although corticosterone does not induce cancellous bone loss in rapidly growing rats, it has been shown to inhibit both cancellous and cortical bone formation in these animals (10, 11, 18). Because an inhibition of bone formation is a common finding in rats subjected to spaceflight (9, 14, 19, 20, 23–27), it follows that corticosteroid excess may play a role in this bone abnormality. However, in the present study, the maintenance of normal indexes of bone formation in the flight rats, despite the occurrence of adrenal hypertrophy, suggests that corticosteroid excess does not inhibit bone formation during spaceflight.

In summary, the present study demonstrates that a relatively long 17-day spaceflight under conditions of group housing has minimal effects on bone mass and turnover in young, rapidly growing, Sprague-Dawley rats. Abundant data from previous studies support the contention that spaceflight decreases bone formation and cancellous bone mass in rats. However, the magnitude of the response of bone to spaceflight appears to be influenced by rat housing. This factor is apparently critical for the planning of future experiments involving use of rats as an animal model for the adverse skeletal effects of spaceflight.

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REFERENCES


