Skeletal Response to Short-Term Weightlessness

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Abstract

Male Sprague Dawley rats were placed in orbit for 7 days aboard the space shuttle. Bone histomorphometry was performed in the long bones and lumbar vertebrae of flight rats and compared to data derived from ground-based control rats. Trabecular bone mass was not altered during the first week of weightlessness. Strong trends were observed in flight rats for decreased periosteal bone formation in the tibial diaphysis, reduced osteoblast size in the proximal tibia, and decreased osteoblast surface and number in the lumbar vertebra. Histologic indices of bone resorption were relatively normal in flight rats. The results indicate that 7 days of weightlessness are not of sufficient duration to induce histologically detectable loss of trabecular bone in rats. However, cortical and trabecular bone formation appear to be diminished during the first week of space flight.

Index Terms: Weightlessness - Trabecular Bone - Bone formation - Osteoblasts - Osteoclasts.

Introduction

Bone loss is a potentially serious consequence of space flight. Skylab astronauts exhibited a 4% decline in the bone mineral density of the calcaneus after 84 days of orbital flight (12). Rats placed in orbit for 18-22 days aboard Soviet Cosmos biosatellites were also characterized by loss of trabecular bone mass (5,17) and decreased breaking strength of the femur and lumbar vertebra (6,13). The osteopenic changes in flight rats appear to be due primarily to diminished bone formation rather than increased bone resorption. For example, an inhibition of periosteal bone formation has been consistently demonstrated by tetracycline labeling techniques in prior Cosmos missions (9,15). A decline in the metaphyseal osteoblast population of flight rats (5) provides additional evidence for diminished bone formation in a weightless environment. On the other hand, the metaphyseal osteoclast population was normal in flight rats (5). Calcium kinetic studies also indicate that bone resorption was not elevated in rats subjected to 18.5 days of weightlessness (3). However, an earlier, transient increase in bone resorption cannot be ruled out.

Current space shuttle missions are normally of 7 days duration. The skeletal effects of such short-term space flight are unknown. In this communication, we report the first histomorphometric analysis of the rat skeleton after 7 days of orbital flight aboard the space shuttle.

Materials and Methods

Experimental details including age, diet, and housing of animals are described by Grindeland et al. (4). Briefly, five large rats (∿400g body weight) and six small rats (∿250g body weight) were placed in orbit for 7 days aboard the space shuttle. Equivalent numbers of large and small rats served as ground-based controls. To label sites of bone formation, calcein (Sigma Co, St. Louis, Mo.) was administered i.p. to all large rats on the 9th and 2nd days prior to launch at a dose of 10 mg/kg body weight. The ground-based control rats were housed at Kennedy Space Center throughout the experiment. After 7 days of orbital flight, the space shuttle landed in California and the flight rats were transported by air to Kennedy Space Center, Fla. This resulted in an 11-17 hour interval between a return to 1G and sacrifice. The large rats were anesthetized with halothane and perfused with a fixative composed of 2% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer. The right tibial shaft, right proximal humerus, and fourth lumbar vertebra were defleshed and placed in 10% phosphate-buffered formalin for 24 hours. The tibial shafts were then dehydrated in ethyl ether and embedded undecalcified in Polyest casting resin (Chemco, San Leandro, Ca.). The portion of the tibial shaft immediately proximal to the tibiofibular junction was sawed into 50 μm-thick cross sections with a Gillings-Hamco thin sectioning machine. The rate of periosteal bone formation was measured with a digital image analysis system interfaced with a Dual System 83 computer (10). The area of bone between the 2 calcein labels was divided by the time interval between their administration to determine the preflight rate of periosteal

bone formation. The area of bone between the second calcein label and the periosteal surface was divided by the time interval between administration of the label and sacrifice to determine the rate of periosteal bone formation during the flight period.

The right proximal humerus and the fourth lumbar vertebra of large rats were dehydrated in ethanol and embedded undecalcified in methyl methacrylate (1). Longitudinal sections (4 μm-thick) were cut with an AO Autocut/Jung 1150 microtome and stained with Masson-Goldner trichrome. The following trabecular bone parameters were measured manually at a magnification of 400X with the aid of a Merz eyepiece reticle (8): trabecular bone volume (%), osteoclast surface (%), osteoblast surface (%), and numbers of osteoclasts and osteoblasts per mm trabecular bone perimeter. Details of data collection and calculations have been described elsewhere (16). In addition, the rate of longitudinal bone growth was measured in unstained, 10 μm-thick sections of the proximal humeral metaphysis and fourth lumbar vertebral body. The distance between the growth plate and the calcein marker administered 2 days prior to launch was measured with a calibrated eyepiece micrometer (14). This distance was divided by the time interval between administration of the calcein marker and sacrifice to calculate the rate of longitudinal bone growth.

The small rats were sacrificed by decapitation. The right tibia and fourth lumbar vertebral body were defleshed and placed in 10% phosphate-buffered formalin for 24 hours. The lumbar vertebra was processed undecalcified and analyzed as described above. The proximal tibia was decalcified in 10% EDTA in Tris buffer, embedded

in paraffin, sectioned longitudinally at 6 μm thickness with a Leitz Wetzlar 1512 microtome, and stained with hematoxylin and eosin. The perimeter of individual osteoblasts was measured in microphotographs of the primary spongiosa with a Zeiss interactive digital analysis system (Carl Zeiss Inc., Thornwood, N.Y.).

Data are expressed as the mean ± SD of control and flight groups. Statistical differences between groups were evaluated with the two-tailed Student's t-test.

Results

Table 1 lists values for the rate of periosteal bone formation in the tibial diaphysis of large flight and control rats. This parameter was nearly identical in the 2 groups during the preflight period. Flight rats exhibited a strong trend for decreased periosteal bone formation during the flight period, but statistical significance was not achieved ($P<0.1$). However, if periosteal bone formation rate during the flight period is expressed as a percentage of periosteal bone formation rate during the preflight period, the value for flight rats (44%) is significantly less ($P<0.05$) than the value for control rats (66%).

Values for bone histomorphometric parameters in the proximal humerus and lumbar vertebra of large flight and control rats are listed in Table 2. Longitudinal bone growth at both skeletal sites was depressed by 15-20% in flight rats, but this trend was not statistically significant. Trabecular bone volume in the proximal humerus and lumbar vertebra was not altered by 7 days of weightlessness. Histologic indices of bone resorption (osteoclast

surface and number) also tended to be normal in large flight rats. On the other hand, these animals exhibited a strong trend for decreased bone formation in the lumbar vertebra, as evidenced by a 50% decrease in osteoblastic parameters. However, this trend is not statistically significant.

Table 3 lists values for bone histomorphometric parameters in the lumbar vertebra of small flight and control rats. Although vertebral trabecular bone volume was decreased by 25% in small flight rats, no statistically significant differences between the 2 groups were observed. The perimeter of individual osteoblasts was measured in the primary spongiosa of the proximal tibial metaphysis. The mean value for small control rats is 23.2 $\pm$ 1.6 μm compared to a mean of 21.2 $\pm$ 1.2 μm in small flight rats. This trend for reduced osteoblast size in small flight rats is not statistically significant ($P<0.1$).

Discussion

The current study indicates that 7 days of weightlessness are not of sufficient duration to induce histologically-detectable loss of trabecular bone in growing rats. In contrast to previous Cosmos studies in which marked trabecular bone loss occurred in rats during 18-22 days of space flight (5,17), trabecular bone volume in the proximal humeral metaphysis and lumbar vertebral body was not significantly altered in rats subjected to a 7-day space flight. An inhibition of periosteal bone formation was consistently observed in prior Cosmos studies (9,15). In the current space shuttle study, flight rats also exhibited a strong trend for decreased periosteal

bone formation. Ground-based control rats had a lower rate of periosteal bone formation in the flight period relative to the preflight period, but this phenomenon was probably age-related. Periosteal bone formation is markedly age-dependent in rats (11). Tetracycline-based evaluations of longitudinal bone growth have never been performed previously in rats subjected to space flight. Our results suggest that weightlessness may suppress longitudinal bone growth, especially in the rapidly-growing long bones. Suppression of periosteal and longitudinal bone growth in a weightless environment would seriously compromise the modeling of a juvenile skeleton.

Previous histologic studies of the skeletal effects of spaceflight were confined to the long bone metaphysis. In the current study, histologic indices of trabecular bone resorption (osteoclast surface and number) were relatively normal in both the proximal humeral metaphysis and lumbar vertebral body of flight rats. This finding is consistent with calcium kinetic and histologic estimates of bone resorption in Cosmos flight rats (3,5). However, several lines of evidence suggest that trabecular bone formation may have been depressed during weightlessness. Osteoblasts tended to be smaller and, presumably, less active in the proximal tibial metaphysis of small flight rats. Furthermore, osteoblastic parameters were decreased by 50% in the lumbar vertebra of large flight rats. This latter finding could be especially meaningful in view of reports that skeletal processes in the rat vertebral column are similar to the remodeling of adult human bone (2).

The question arises as to whether the 11-17 hour interval between a return to 1G and sacrifice affected the experimental results. This

time period is not of sufficient duration to have major effects on parameters such as trabecular bone volume, periosteal bone formation, or longitudinal bone growth. However, the bone cell population may have responded to the return to 1G. The cell cycle time of immature osteoblast precursors is 36-48 hours (7,18), which indicates that these cells would not have had enough time to contribute to an increase in the osteoblast population. On the other hand, differentiation of mature osteoblast precursors to increase the osteoblast pool may occur more rapidly. Since small flight rats were found to have greater numbers of mature osteoblast precursors than large flight rats (W.E. Roberts, unpublished results), these animals may be expected to increase their osteoblast numbers more rapidly after a return to 1G than large flight rats. The finding that vertebral osteoblastic parameters were reduced by 50% in large flight rats whereas the same parameters in small flight rats were nearly identical to control values may be explained on this basis.

In summary, the current study demonstrates that histologically-detectable bone loss does not occur in growing rats during the first week of space flight. However, large flight rats exhibited strong trends for an inhibition of cortical and trabecular bone formation. These findings are consistent with the hypothesis that the primary skeletal alteration induced by space flight in growing rats is diminished bone formation.

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TABLE 1. The rate of periosteal bone formation in the tibial diaphysis of large flight and control rats (~400 g BW).

	RATE OF PERIOSTEAL BONE FORMATION (PBFR) ($\times 10^{-3}$ MM^2/DAY)		
	PREFLIGHT PERIOD	FLIGHT PERIOD	FLIGHT PBFR/PREFLIGHT PBFR (%)
CONTROL (N=5)	29.9 ±3.6	19.9 ±6.6	66.0 ±16.1
FLIGHT (N=4)	30.1 ±2.5	13.2 ±2.4	43.7[a] ±6.5

All values are mean ± SD.

[a] $P<0.05$

Table 2. Bone histomorphometric parameters in large flight and control rats (∿400g BW).

	LONGITUDINAL BONE GROWTH (μm/day)	TRABECULAR BONE VOLUME (%)	OSTEOCLAST SURFACE (%)	OSTEOBLAST SURFACE (%)	OSTEO-CLASTS/MM	OSTEO-BLASTS/MM
Proximal Humerus						
Control (N=5)	91.2 ±18.0	7.2 ±5.1	14.6 ±3.7	30.5 ±6.1	4.9 ±1.3	20.5 ±3.0
Flight (N=4)	74.2 ±13.3	6.3 ±2.2	17.2 ±4.6	25.6 ±6.1	5.6 ±1.6	17.2 ±3.3
Lumbar Vertebrae						
Control (N=5)	14.9 ±4.7	18.3 ±7.0	5.9 ±1.3	9.5 ±3.4	2.2 ±0.5	6.0 ±2.0
Flight (N=4)	12.6 ±3.7	22.4 ±5.7	7.2 ±5.0	4.7 ±5.0	2.2 ±0.6	2.9 ±3.2

All values are the mean ± SD.

TABLE 3. Histomorphometric parameters in the lumbar vertebrae of small flight and control rats (~250 g BW).

	TRABECULAR BONE VOLUME (%)	OSTEOCLAST SURFACE (%)	OSTEOBLAST SURFACE (%)	OSTEOCLASTS/MM	OSTEOBLASTS/MM
CONTROL (N=6)	20.8 ±5.9	11.1 ±4.6	19.0 ±4.7	3.4 ±1.2	12.4 ±3.7
FLIGHT (N=6)	15.0 ±5.8	10.2 ±2.5	18.2 ±5.6	3.3 ±0.9	11.3 ±3.6

All values are the mean ± SD.