EXPERIMENT K-6-04

TRACE ELEMENT BALANCE IN RATS DURING SPACEFLIGHT

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INTRODUCTION

Exposure to microgravity causes alterations in the skeletal and mineral homeostatic systems. Decreased mass of bone can be explained by decreased formation, increased resorption or a combination of processes. Previous investigations have shown that in young growing rats, diaphyseal bone formation is decreased (1). Other evidence shows that bone resorption is also decreased, apparently secondary to a decrease in total body calcium turnover (3); this is evidenced systemically even in the non-weight-bearing bones of the jaw (3). In young rats exposed to only 7 days of flight, however, bone composition was not significantly affected (4). This overall slowing of bone turnover, the dynamics of calcium excretion (2) and differential effects on bone seen in flights of varying duration suggest that, at least in the younger growing rat, a variety of different metabolic processes may be affected in addition to bone formation and resorption.

Little is known about the effects of flight in an older skeleton; limited data suggest that bone resorption is increased after 5 days (5) but no data are available about other metabolic effects. The response of a more slowly-growing skeleton to microgravity may be different than that of a younger animal, similar to the different responses seen in adolescents and adult humans to immobilization (6). This experiment was designed to investigate changes occurring in skeletal and mineral homeostatis in these older rats flown for two weeks in space.

METHODS AND RESULTS

Vertebral specimens from a total of twenty rats were obtained for analysis (five each from basal control, flight, synchronous control, and vivarium control groups). In addition, a pooled excreta collection from each group and samples of the flight paste diet were received for analysis. The vertebrae (fourth lumbar) were dissected free of surrounding tissues using titanium tools to minimize possible elemental contamination. Each vertebra was weighed, then separated into four parts. The vertebral body was dissected free of the posterior elements at the base of the pedicles, then each of these components was split into two parts for analysis.[1] The results in this report include analyses to date of major constituent elements and osteocalcin.

Separated bone specimens were lyophilized to constant weight, then ground to a fine powder in a liquid-nitrogen-cooled mill. Osteocalcin content was measured in EDTA extracts of the powder using the method of Patterson-Allen et al (7) with a rat-specific osteocalcin assay. Calcium was measured using atomic absorption spectrophotometry, and phosphorus was determined using a modified Fiske-Subarow method. The results of these assays are given in Table 1. Derived quantities are given in Table 2.

DISCUSSION

The spine is composed of two types of bone. The trabecular portions of the vertebral body as well as the compact shell in this region are in direct contact with bone marrow and can be very responsive to metabolic stimuli. In contrast, the posterior elements containing many muscle attachments are primarily compact bone with a slower turnover rate. In humans and non-human primates, significant vertical loading forces exist in the spine during normal weightbearing and much of this force is transmitted through the vertebral body which makes up about half of the

[1] The original experiment design called for constituent analysis to include both major and trace elements and bone osteocalcin content. However, between design of the experiment and receipt of samples the technique to be used for trace analysis in these small samples (neutron activation analysis) became unavailable because of closure of the nuclear reactor at the University of California, Berkeley. At the present time, development and testing of new techniques sensitive enough to do this analysis are underway (using high-sensitivity x-ray fluorescence spectrometry) and will be used within the next six months to complete the sample analysis. These data will be included in a supplemental report at that time.

vertebral mass. In rats, the posterior elements take up much more of the loading forces at 1-g as well as the torsional forces due to muscle pulls, and are significantly different in their morphology than in primates, comprising about two-thirds of the total vertebral mass. Thus, we may expect that the two portions of the rat vertebra, the vertebral body and the posterior elements, will show different responses to spaceflight.

The results of the analyses from this study confirm major differences between portions of the vertebra. The posterior bone is more highly mineralized, evidenced by increased concentration (per unit weight of bone) of calcium (5%), phosphorus (6%) and osteocalcin (37%), similar to the differences seen between proximal and mid humerus in previous studies (4). The major increase in osteocalcin content indicates the presence of mature, low-turnover bone.

The differences between flight and control animals were minimal in these older, slowly-growing rats. Mass of whole vertebrae increased 6.2% in synchronous rats compared to less than 2% in flight rats over the 16 days when compared to basal controls, suggesting a decreased rate of bone growth in flight. Compared to young rats in which vertebral mass increased over 40% in 10 days in controls and 20% in flight rats (5), this may be a clear indication that even in the older skeleton bone growth will slow in microgravity. The increased osteocalcin concentration in the posterior spine of flight rats compared to all other groups suggests a higher state of maturation of this compact bone, possibly due to a slowed turnover with the removal of both dorsal-to-ventral loading as well as torsional muscle pulls in spaceflight. This is similar to the differential effects of long-term microgravity exposure seen on the vertebral body and posterior elements in cosmonauts (8), and suggests further study of the muscle-spine interaction is necessary to determine the actual effects of spaceflight and changed patterns of activity and loading on the spine.

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TABLE 1. MAJOR CONSTITUENTS OF LUMBAR VERTEBRAE

	Basal	Vivarium	Synchronous	Flight
Wet Weight (mg) Whole Vertebra	189.4±6.6	207.6±5.9	201.1±4.0	193.0±7.4
Vertebral Body Wet Weight (mg)	66.1±3.1	86.3±3.1	66.0±4.1	67.3±4.1
Vertebral Body Water Content (%)	36.4±0.8	32.6±0.8	34.9±0.9	40.0±1.9[a]
Posterior Element Water Content (%)	30.8±1.2	28.4±1.6	29.9±1.0	27.2±1.9
Ca (mg/gm) Vertebral Body	203.5±4.7	220.0±4.7	217.1±2.7	213.1±0.6
Ca (mg/gm) Posterior Elements	217.3±1.2	224.8±3.3	222.9±5.0	233.7±3.4
P (mg/gm) Vertebral Body	110.3±2.1	115.3±2.1	114.2±1.4	115.0±2.1
P (mg/gm) Posterior Elements	119.4±3.8	116.9±5.3	119.7±5.3	123.5±1.0
Osteocalcin (mg/gm) Vertebral Body	1.72±0.07	2.05±0.07	1.74±0.12	1.77±0.12
Osteocalcin (mg/gm) Posterior Elements	2.42±0.01	2.46±0.07	2.42±0.07	2.61±0.08

TABLE 2. DERIVED MEASUREMENTS FROM ANALYSIS OF VERTEBRAE (MEAN±SEM)

	Basal	Vivarium	Synchronous	Flight
Vertebral Body % of Total Vertebra	35.0±1.0	41.8±2.1	32.7±1.6	35.0±2.3
Ca/Pi (Molar) Vertebral Body	1.43±0 04	1.48±0.04	1.47±0.03	1.44±0.02
Ca/Pi (Molar) Posterior Elements	1.41±0.03	1.49±0.01	1.47±0.04	1.47±0.01
OC/Ca Vertebral Body	8.53±0.53	9.34±0.54	8.02±0.49	8.32±0.55
OC/Ca Posterior Elements	11.14±0.05	10.93±0.33	10.90±0.42	11.16±0.30