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Effects of Spaceflight on Structural and Material Strength of Growing Bone (41729)

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Abstract. Rats in space for 18.5 days did not exhibit the normal gain in femoral bone strength of terrestrial controls. The strength deficit may have been caused by multiple factors including a diminished bone formation and an inhibition of the gain in tissue material strength. Centrifugation at 1*g* in space substantially enhanced bone strength, possibly by promoting more normal tissue maturation. Full recovery of bone strength was achieved 25 days after reentry.

Astronauts and cosmonauts have shown changes in calcium homeostasis and tendencies toward a decreased bone mass after prolonged spaceflight (1, 2). When growing rats were orbited for 19 days aboard Cosmos 782, the periosteal bone formation rate in the tibial diaphysis was decreased, as was the femoral breaking strength (3, 4). Cosmos 936 offered the opportunity to determine whether centrifugation at 1*g* during flight might deter such changes in bone. To test this hypothesis, we determined the torsional strength of femora in rats that had been flown for 18.5 days aboard this spacecraft.

Materials and Methods. Forty male, 63-day-old, Czechoslovakian Wistar rats weighing an average of 202 ± 13.9 g were divided into four groups: two flight and two control. The flight groups consisted of stationary (noncentrifuged) flight rats and centrifuged flight rats. The ground-based control groups were composed of vivarium control rats and simulated-flight control rats (5). The simulated-flight control experiment was initiated 4 days after launch so that environmental parameters could be made to mimic closely those telemetered from the spacecraft. The noise level was raised to 110 db and a vibration frequency of 50–70 Hz at an amplitude of 0.4 mm was applied to animal holding units for 10 min. Immediately following noise and vibration stresses, animals were subjected to acceleration for a period of 10 min with a plateau of 4*g* for 7 min. During the flight control experiment, the quality of cabin air and the environmental temperature were regulated to simulate that of the orbiting spacecraft. After completion of the control experiment, renetry stresses were applied to the animals. They were first accelerated for 5 min with a plateau of 6*g* for 3 min and subsequently subjected to an impact shock with a magnitude of 50*g* and a duration of 10 msec. Following the application of reentry stresses, the animals, as well as all other biological specimens, were handled exactly as the flight specimens. Six rats were sacrificed at 63 days to serve as basal controls for the flight and vivarium groups, and six additional rats were sacrificed at 67 days to serve as basal controls for the simulated-flight control group. Cosmos 936 contained two centrifuges, each with five cages. Each centrifuge had a radius of 32 cm (from the centrifuge center to the longitudinal axis of the animal during rotation) and rotated at 53.3 ± 3 rpm. This rotation rate provided an acceleration of 1*g* to the animals. The centrifuges were switched on when the spacecraft reached its orbit and were switched off about 5 hr before landing. Animal care, as well as preflight and postflight activities, was controlled by the Soviets, as described previously (5).

Half of the flight rats and the control rats were sacrificed at the end of the 18.5-day flight, whereas the remaining animals were sacrificed 25 days after reentry. After sacrifice, one femur from each animal was carefully removed allowing muscle tissue to adhere to the bone. The femur, with attached muscle, was placed into a Zip-Lock bag and frozen on dry ice. After all of the bones were removed, the bags were placed into separate vials (one bag/vial)

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in a dry ice transport container. During transit, the bones remained frozen between −10 and −30°C. The bones were kept frozen until they were analyzed. After thawing to room temperature, the bones were maintained in a moistened condition at all times. Femoral bone densities were measured by trichloroethylene displacement in a volumetric pycnometer (6). The ends of the left femur were embedded in an acrylic resin, and a standard torsion test machine was used to determine the mechanical properties (7, 8). The bones were loaded to failure with a total time to fracture of approximately 0.1 sec (8). A curve of the applied torque versus angular displacement was recorded on a storage oscilloscope for each bone tested. These curves were later used to establish the ultimate torque, torsional stiffness, energy absorbed to failure, and angular deformation to failure (7, 8).

Because the femora were broken by mechanical testing, we were not able to make accurate accounts of femoral cross-sectional geometry and bone formation during the experimental period. Instead, we measured the medullary area and the bone area from thin sections cut from the left tibiae of the rats according to procedures described previously (3). These bones had been transported to the United States and subsequently handled in the same manner as were the femora. Although the bone cross-sectional area of the rat tibia is less than that of the femur, bone formation in the two bones has previously been shown to be similar (9, 10). In addition, Wronski and Morey have shown that orbital flight induced a significant inhibition of periosteal bone formation in both the tibial and humeral diaphyses of growing rats (11).

Results. Spaceflight had a dramatic effect on the torsional mechanical properties of the growing rat femora and on bone growth (Ta-

TABLE I. TORSIONAL PROPERTIES OF RAT FEMORA

	N	Torque[a] (Ncm)	Stiffness[a] (Ncm/rad)	Energy[a] (mJ)	Deformation[a] (degrees)
Basal values					
First basal group (63-day-old rats)	6	22.5 ± 3.1	318 ± 78	29 ± 10	14.2 ± 4.1
Second basal group[b] (67-day-old rats)	6	20.6 ± 4.2	310 ± 59	24 ± 12	12.4 ± 4.0
Values obtained after 18.5-day spaceflight					
Vivarium control group	5	32.2 ± 9.6*	482 ± 72*	37 ± 20	11.4 ± 3.3
Simulated-flight control group	5	26.1 ± 6.9	415 ± 39*	26 ± 13	11.8 ± 2.6
Stationary flight group	5/4[c]	17.5 ± 4.4	297 ± 22	18 ± 9	10.3 ± 2.2
Centrifuged flight group	5/4[c]	29.2 ± 1.5*	433 ± 44*	33 ± 5*	13.1 ± 2.0
Values obtained 25 days after reentry					
Vivarium control group	5	39.2 ± 9.2	496 ± 79	50 ± 31	13.8 ± 5.3
Simulated-flight control group	5	35.9 ± 4.4*	476 ± 72	42 ± 13	13.5 ± 2.7
Stationary flight group	5/4[c]	43.9 ± 3.6	500 ± 53	59 ± 9	15.7 ± 2.7
Centrifuged flight group	5	37.9 ± 7.6	537 ± 49	46 ± 22	15.7 ± 2.7

[a] Means ± SD.

[b] This group provided baseline measurements for the simulated-flight control experiment which was begun 4 days after launch so that environmental parameters could be made to mimic closely those telemetered from space.

[c] Although bones from five rats were received, only four bones were analyzed because one rat starved due to a feeding system malfunction and two bones were broken prior to analysis.

* Significantly different from corresponding value for stationary flight group ($P < 0.05$).

bles I and II). During the 18.5 days in space, the stationary flight group demonstrated no increase in torsional strength compared with basal controls. By contrast, vivarium and simulated-flight control rats sacrificed after 18.5 days demonstrated a gain of approximately 35% in failure torque over the basal controls. The stationary flight rats had significantly lower reentry values of failure torque, stiffness, and energy than either vivarium controls or flight-centrifuged rats (Table I). In addition, the tibial bone area of the stationary flight rats was only slightly above the value for the basal controls and was significantly less than that for the vivarium controls (Table II). By 25 days after reentry, the mechanical properties had been restored to normal or above normal, and bone growth slightly exceeded that in the vivarium controls (Tables I and II).

The femoral mechanical properties of the rats that were centrifuged during flight were not significantly different from those of vivarium or simulated-flight control rats, even though the centrifuged animals weighed significantly less than the animals in either control groups (Tables I and II). Centrifugation in space thus facilitated the more normal gain of bone structural properties in growing rats. The finding presents an interesting paradox inasmuch as centrifugation did not appear to prevent a deficit in bone growth; at reentry, tibial cross-sectional geometries were only slightly above those in the stationary flight rats (Table II).

Possible explanations for the deficit in bone mechanical properties exhibited by the stationary flight rats were differences in geometry, differences in bone material properties, or both. Since periosteal bone formation had been found to decrease during spaceflight in the present investigation (Table II) and in previous studies (3), it was reasonable to suspect differences in geometry as the primary cause of the loss of bone strength. To test this hypothesis, we generated hollow elliptical stress analysis models (12) of the vivarium control femora and the stationary flight rat femora. Measurements of the geometries of femora

TABLE II. GROWTH DATA

		Femur		Tibia	
	Animal weight[a] (g)	Density[a] (g/cm^3)	Length[a] (cm)	Medullary area[a] (mm^2)	Bone area[a] (mm^2)
Basal values					
First basal group (63-day-old rats)	209 ± 21	1.42 ± 0.03	3.12 ± 0.10	0.78 ± 0.36	2.90 ± 0.14
Second basal group[b] (67-day-old rats)	235 ± 15	1.40 ± 0.08	3.17 ± 0.08	0.80 ± 0.36	2.96 ± 0.23
Values obtained after 18.5-day spaceflight					
Vivarium control group	289 ± 9	1.60 ± 0.02	3.51 ± 0.08	0.90 ± 0.04	3.68 ± 0.40
Simulated-flight control group	294 ± 16	1.60 ± 0.01	3.44 ± 0.06	1.06 ± 0.14	3.34 ± 0.16
Stationary flight group	295 ± 29	1.56 ± 0.05	3.41 ± 0.08	0.97 ± 0.15	3.01 ± 0.21*
Centrifuged flight group	264 ± 8	1.61 ± 0.01	3.39 ± 0.03*	1.07 ± 0.17	3.11 ± 0.07*
Values obtained 25 days after reentry					
Vivarium control group	332 ± 8	1.61 ± 0.02	3.59 ± 0.03	0.90 ± 0.15	3.65 ± 0.34
Simulated-flight control group	337 ± 14	1.62 ± 0.03	3.61 ± 0.07	0.88 ± 0.14	3.89 ± 0.23
Stationary flight group	326 ± 15	1.63 ± 0.03	3.59 ± 0.05	1.08 ± 0.15	3.89 ± 0.17
Centrifuged flight group	351 ± 17	1.63 ± 0.03	3.62 ± 0.09	1.01 ± 0.24	3.94 ± 0.26

[a] Mean ± SD.
[b] See explanation, Table I.
* Significantly different from corresponding value for vivarium controls ($P < 0.05$).

were taken from rats whose weights were comparable to the mean weight of the vivrium control rats at reentry. The vivarium control model had a major axis of 3.98 mm, a minor axis of 2.97 mm, and a medullary area of 4.66 mm^2. The cortical bone area was 4.64 mm^2. With this model, using an ultimate failure torque of 32.2 Ncm that simulated the mean reentry value for the vivarium control rats, we calculated a bone tissue shear strength of 61.7 MPa. This value was consistent with literature values for adult human cortical bone shear strength (13).

In constructing the model for the stationary flight rats, we assumed that the medullary area was identical to that for the vivarium control rats (3, 1) (Table II). Our calculations of the major and minor axes of the femoral cross section in this model, however, were based on the premise that the mean failure torque of 17.5 Ncm for the stationary flight group reflected differences in geometry only (not in tissue shear strength) with respect to the vivarium controls.

The results of the stress analyses in the stationary rat femur model indicated that the bone area of the stationary flight rat would have had to have been 2.44 mm^2 (47%) less than that of the vivarium control rat if decreased bone geometry had been the sole cause of the deficit in mechanical properties in the stationary flight group. The tibia data indicated, however, that this value significantly exceeds the total amount of bone that could have been deposited during the 18.5-day spaceflight period. In fact, the total tibial bone formed in the mean vivarium control rat over the 18.5-day period was only 0.62 mm^2, or 18% of the total bone area (Table II). Correspondingly, the tibial cross-sectional area of the stationary flight rat was 0.67 mm^2 (18%) less than that of the vivarium control (Table II). Therefore, insufficient bones was formed during the 18.5-day period to allow the strength differences to be attributed solely to differences in geometry. Furthermore, bone cross-sectional geometries of the stationary flight rats were only slightly smaller than those of the centrifuged flight rats (Table II), even though structurally the bone was significantly weaker in the stationary flight group (Table I).

Discussion. Our results suggest that in addition to differences in geometry, significant differences in bone material properties between the stationary flight rats and both groups of control rats must have existed. An abnormal maturation of the mineralized tissue may have caused the bone to be weaker in the stationary flight rats than in the controls. The centrifuged flight rats showed only a slight (not statistically significant) deficit in femoral structural properties compared with vivarium controls, despite the fact that periosteal bone growth was impaired. The impaired bone growth in the centrifuged animals may have resulted from difficulty in adapting to the short radius centrifuge and/or inadequacy of centrifugation to precisely simulate 1*g* at earth. Although centrifugation could not prevent the skeletal growth deficit, it did facilitate normal tissue maturation and the attainment of normal tissue properties.

The hypothesis that bone tissue maturation may be impaired in the stationary flight animals but not the centrifuged flight animals suggests differences in bone density and/or bone composition between the two groups. The basal control femora had a density value of 1.42 ± 0.03 g/cm^3 (Table II), which is significantly less than the reentry density values of about 1.60 g/cm^3 for the vivarium controls, simulated-flight controls, and centrifuged flight rats. The stationary flight rat femora had a density of only 1.56 ± 0.5 g/cm^3 This difference, although not statistically significant, is notable. Perhaps more important to the hypothesis are the results of the density fractionation studies by others that indicate that spaceflight causes a delay in the normal maturation of bone mineral and matrix (14). In the rat mandible, spaceflight of 18.5 days resulted in larger than normal proportions of Ca, P, and hydroxyproline in the 1.3–1.9 density fraction and less than normal proportions in the 2.2–2.9 density fraction. These data support our inference that tissue material properties were deficient in the stationary flight rats but not in the centrifuged flight rats. The recovery of mechanical properties in the 25-day period after spaceflight may be due to a recovery of both bone geometry (Table II) and tissue maturation.

The weightlessness of the space environ-

ment may have more pernicious effects on the bones of maturing animals than does a simple reduction of activity in an earth environment. The exercise activity of immature swine on earth has been shown to have a profound influence on the quantity, but not the quality, of bone produced (15). Our data indicate that in growing animals both bone quantity and quality are impaired during spaceflight. The bone quality, however, may be spared by centrifugation. In view of the planned increase in space shuttle travel, further studies with animal species having more mature skeletons are needed to discern the possible implications of these findings for human space travel.

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