Space-related bone mineral redistribution and lack of bone mass recovery after reambulation in young rats

MARIE-HÉLÈNE LAFAGE-PROUST, PHILIPPE COLLET, JEAN MICHEL DUBOST, NORBERT LAROCHE, CHRISTIAN ALEXANDRE, AND LAURENCE VICO
Laboratoire de Biologie du Tissu Osseux, Groupement d’Intérêt Public Exercice, Faculté de Médecine, 42023 Saint-Etienne, France

Lafage-Proust, Marie-Hélène, Philippe Collet, J ean Michel Dubost, Norbert Laroche, Christian Alexandre, and Laurence Vico. Space-related bone mineral redistribution and lack of bone mass recovery after reambulation in young rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R324–R334, 1998.—This study reports the effects of a 14-day spaceflight followed by a 14-day reambulation period on bones of 56-day-old male rats compared with synchronous (S) and vivarium (V) control animals. Femur, tibia, and humerus bone mineral densities (BMD); bone calcium and phosphorus concentrations ([Ca2+] and [P]), measured by X-ray microanalysis (XRM), on tibia, vertebrae, and calvaria; and histomorphometric data on proximal primary and secondary spongiosae (I and II SP, respectively) of the tibia and humerus were measured. After the flight in flown rats (compared with S), BMD was lower in the distal femur and remained similar to S in humerus and tibia, [Ca2+] and [P] were lower in tibia II SP and higher in calvaria, tibia I SP width and II SP bone volume were lower, resorption was markedly higher in tibia II SP, and no difference in formation parameters was observed. After reambulation, BMD was lower in long bones of both flight and S groups compared with V. Bone loss appeared in humeral II SP and worsened in tibial II SP in flown rats. Tibial formation parameters were higher in flown rats compared with V and S, indicating the onset of an active recovery. Tibial XRM [Ca2+] and [P] in flown rats remained below control levels.

X-ray microanalysis; histomorphometry; densitometry; microgravity

MICROGRAVITY AND SPACE-RELATED conditions induce bone loss of both cortical and trabecular envelopes, essentially in weight-bearing bones. This bone loss is mainly due to decreased bone formation (14, 29). Bone resorption has been found to be either unchanged (8) or increased (9, 26), according to the duration of the space mission. We (26, 29) and other authors (8) have studied the bone cellular changes after 1, 2, and 3 wk of Biocosmos spaceflights. In that series, the secondary spongiosa (II SP) of tibial metaphysis of male Wistar rats of approximately the same weight (300–350 g) and killed at approximately the same time after landing was analyzed. During the first week, bone loss was due to decreased bone formation in II SP combined with decreased supply from the primary spongiosa (I SP). During the second week, bone loss persisted because of the abrupt and transient increase in bone resorption combined with a trend toward a decrease in osteoid parameters. Finally, after the third week, the results were indicative of normal or slightly reduced bone resorption with a persistent decrease in bone formation parameters. The bone volume occupied by I SP was also found decreased after 7- (9, 29), 14- (10, 26), and 22-day (31) spaceflights. These results, along with the chondrogenic anomalies observed in growth plate cartilage (13), were indicative of an abnormal endochondral ossification process. Physicochemical analyses provided evidence of a reduction of bone mineral content, hydroxypatite crystal size, stiffness, and strength of long bones in flight groups (12, 16, 25). These results suggested the existence of a bone mineralization defect affecting different regions to varying degrees.

Although spaceflight-induced bone loss is starting to be more clearly elucidated, the modalities of bone mass recovery on earth still remain unclear. In humans, measurements performed up to five years after a Skylab mission showed that calcaneum bone mineral content remained decreased (23). Rats from the Cosmos 605 (31) and Cosmos 1129 (8) missions exhibited incomplete bone mass recovery after a reambulation period lasting longer than the flight itself.

In this context, we studied skeletal changes immediately after a 14-day flight and after a 14-day recovery period in various bones of male Sprague-Dawley rats that participated in the space mission. This 2-wk mission allowed verification of the trends toward decreased tibial bone formation and a more precise characterization of bone cellular responses to reloading after microgravity exposure. Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DEXA) in total humerus, femur, and tibia, and histomorphometric analysis of tibial and humerus proximal metaphyses was performed. Ca2+ and P concentrations and Ca2+/P molar ratio were evaluated in both cancellous and cortical compartments of tibia, vertebrae, and parietal bones using X-ray microanalysis.

MATERIAL AND METHODS
STS 58–SLS2 Mission

This National Aeronautics and Space Administration (NASA) mission consisted of a 14-day spaceflight followed by a 14-day recovery period for some animals. Rats were transferred to the Research Animal Holding Facility (RAHF) 2 days before launch. RAHF is a rodent flight facility for experiments to be conducted in the Spacelab, providing food and water ad libitum, waste management, and light, temperature, and airflow control. Animals were individually housed in transparent polycarbonate cages especially developed for the flight (10 × 11 × 26 cm). All cages had wood shaving bedding. Rodents were maintained on Purina rat chow from arrival at the NASA laboratory until the day of launch (18 October 1993), when they were switched to the food bar diet with the same composition as Purina chow. Food and water...
were supplied ad libitum. During the flight, animals were exposed to a 12:12-h light-dark cycle and a temperature of 25 ± 1°C. During the recovery period, rats had a normal reambulation and were fed with the same paste diet as before the flight.

Rat Groups

Thirty male Sprague-Dawley rats from Taconic Farms were randomized to six groups. At the beginning of the mission, the rats were 56 days old and weighed 209 ± 14 g. The following groups of five rats each were available: 1) basal control group providing a preflight control killed on the day of the launch (body weight = 251 ± 10 g) (C0), 2) flight group killed around the time of landing (body weight = 315 ± 12 g) (F14), 3) flight-synchronous group kept under conditions identical to those of the spaceflight group except for microgravity (body weight = 317 ± 12 g) (S14), 4) flight plus recovery group (body weight = 373 ± 13 g) (F+R28), 5) flight-synchronous plus recovery group (body weight = 358 ± 13 g) (S+R28). Animals were exposed to the same conditions as flight + recovery group except for microgravity. 6) Recovery control group is a vivarium group killed at the end of the recovery period (body weight = 387 ± 14 g) (C28).

Sample Preparation

Animals were killed by decapitation 2–3 h after landing for the flight and flight-synchronous groups and at the end of the recovery period for the flight + recovery and flight-synchronous + recovery groups. The skull, last thoracic vertebra, right humerus, right femur, and right tibia were removed, cleaned, and fixed in 10% phosphate-buffered formaldehyde solution. The right humerus, right femur, and right tibia were removed, cleaned, and fixed in 10% phosphate-buffered formaldehyde solution. These samples were placed in 1 cm high acetone and sent to our laboratory under these conditions.

Morphological Procedures

The length of each long bone was assessed in its long axis using calipers (precision: 0.01 cm). Three repeated measurements were performed for each sample.

Densitometric Measurements

BMD (g/cm²) was evaluated by DEXA (Hologic QDR-1000; Hologic, Paris, France) using an HR-1 high-resolution scan module for small animal applications. The femur, humerus, and tibia were measured ex vivo. Samples were placed in 1 cm of water to simulate soft tissues (1). Reproducibility was evaluated by five nonconsecutive measurements of each bone type. The BMD coefficient of variation was 0.70% for the proximal humerus, 0.33% for the tibia, and 2.81% for the femur (28). A good correlation between measurements obtained with a laboratory and those obtained with conventional biochemical analysis had been observed (ash weight and Ca²⁺ content r² = 0.97) (28).

X-Ray Microanalysis

X-ray microanalysis was performed according to a previously published method (4). Briefly, methyl methacrylate-embedded bone blocks were polished in the frontal plane for the tibia (after histomorphometric processing, see below), the transverse plane through the centrum of vertebrae, and the sagittal plane for parietal bones. Blocks were then made electrically conductive by coating them in a vacuum evaporator with a 50-nm layer of carbon (SCD 040; Balzer Union, Basel, Switzerland).

Examination. Energy-dispersive X-ray microanalysis was performed using a J EOL J SM 840 scanning electron micro

scope (J EOL, Tokyo, Japan) equipped with a TRACOR Si(Li) energy-dispersive X-ray spectrometer. The operating conditions were as follows: accelerating voltage = 15 kV, beam current = 1 nA, take-off angle = 40°. Spectra were collected by point electron beam at x400 for 50 s live time.

Quantitative analysis. The peak-to-background ratio method was used to measure the Ca²⁺ and P concentrations in bone samples (11). Corrections for absorption and atomic number (Z²/A) were included. CaCl₂ and KH₂PO₄ processed in a similar way as bone samples (fixation, dehydration, embedding) were used as standards. The concentration of element x in the specimen (Cₓ) was calculated according to the formula

\[
C_x = \frac{(I/W)_{sp} \times (Z^2/A)_{sp}}{(I/W)^{st} \times (Z^2/A)^{st}} \times C_x^{st}
\]

where Cₓ is the concentration of the element x in moles per kilogram; I/W is the peak-to-background ratio for the element x; the subscripts sp and st refer to specimen and standard, respectively; and the value Z²/A is the mean value of the squared atomic number divided by atomic weight in the sample.

Calcium and phosphorus concentrations were measured on different bone compartments. Three sites were distinguished in the proximal tibia: cortical bone, I SP, and II SP. Two regions were analyzed in the vertebral body: cortical bone and II SP. No site distinction was made in parietal bone, in which only cortical bone was analyzed.

Qualitative analysis. To estimate bone apatite heteroionic substitutions, the Ca²⁺/P molar ratio was calculated on the same regions as the concentrations. The Ca²⁺/P molar ratio of stoichiometric hydroxyapatite is 1.66. Synthetic hydroxyapatite was used as control. This control was embedded in pure methyl methacrylate under the same conditions as bone samples.

Histomorphometry

Cancellous bone measurements were performed on the humeral and tibial proximal metaphyses and were expressed according to international nomenclature (15). An area adjacent to Sharpey's fiber in the greater trochanter of the proximal femur was also measured (29). A Leitz-TAS automatic image analyzer equipped with a Bosch camera connected to a Leitz Orthoplan microscope (Leica, Lyon, France) was used to determine the bone volume (bone volume/tissue volume, percent of cancellous bone area) and structural indexes (trabecular bone thickness (TbTh) and number (TbN)) in both I SP and secondary II SP spongiosae. These measurements were performed on six modified Goldner sections (29). To compensate for any differences in longitudinal growth between groups, the region of interest representing II SP was delineated anatomically from the bottom of I SP to a zone located proximal to the diaphysis/metaphysis border. Trabecular number decreases with distance from the growth plate, suggesting that bone formation decreases and resorption increases when the analysis is performed closer to the diaphysis. The static bone cellular parameters (fluorochrome labeling was not available) were measured in the II SP using a semiautomatic system composed of a digitizing table (Summasketch; Summagraphics, Marseille, France) connected to a personal computer and a Reichert Polymar microscope (Reichert, Darmstadt, Germany) equipped with a drawing system (camera lucida). These measurements included the osteoid surfaces (osteoid surface/bone surface, %) as the length of osteoid seams covering bone-forming surfaces on four Gol

ner's sections, osteoblastic surfaces (osteoblastic surface/bone
surface, %) on four Toluidine blue-stained sections, osteoclast number (cells/mm of trabecular bone), and active resorption surfaces (osteoclast surface/bone surface, %) as the length of osteoclastic cells covering bone-resorbing surfaces determined on four sections stained for tartrate-resistant acid phosphatase activity.

The longitudinal growth rate was assessed indirectly (27) by measuring the I SP width at four equidistant points between the lower limit of the growth plate cartilage and the limit between the primary and secondary spongiosae (when trabeculae were no longer parallel and became independent).

Statistical Analysis

All results are presented as medians (25th-75th percentiles) due to distribution-free data. Nonparametric analysis of variance (Kruskal-Wallis test) was used to test differences between the six groups. The Mann-Whitney test for comparison of two samples was used to compare specific variables between any two of the six groups. Values of P ≤ 0.05 were considered to be statistically significant.

RESULTS

Bone Length

Bones were longer in C28 than in C0. No difference was observed between F14 and S14 or between F + R28, S + R28, and C28. F + R28 femur and tibia were longer than that of F14, and the S + R28 femoral and humeral lengths were greater than in S14 (Table 1).

BMD

Effect of growth. BMDs of C28 femur, tibia, and humerus were greater than those of C0. These rats therefore continued to accumulate bone minerals. However, except in the distal and middle femoral subregions, this increment was not uniformly distributed within the same bone: in the tibia, the BMD increase of the metaphyseal region was less than that of the diaphysis, whereas the opposite was observed in the humerus (Table 2 and Fig. 1).

Effects of the 14-day spaceflight. At the end of the spaceflight, no change in BMD in any region was seen between F14 and S14 in either the tibia or the humerus. Detectable bone loss was observed in the femoral metaphyseal region in F14 versus S14 (Fig. 1).

Effects of a 14-day reambulation period after the spaceflight. After a reambulation period of similar duration to that of the space mission, BMDs of all regions measured in the F + R28 and S + R28 groups were significantly decreased, except for the femoral and humeral diaphyseal BMDs in S + R28, compared with C28. This suggests that the long bones of F + R28 and S + R28 did not accumulate minerals at the same rate as the vivarium controls. However, the F + R28 BMD was significantly greater than that of F14 in the following sites: diaphysis (for all samples), entire femur and humerus, and distal femur, indicating an active recovery process. In the proximal humerus and tibia, no difference was observed between F14 and F + R28 and no difference was observed between F + R28 and S + R28 BMDs (except for the entire and middle femur), suggesting that flight simulation conditions affected bone BMD to the same degree as spaceflight (Table 2 and Fig. 1).

X-Ray Microanalysis

Ca2+ and P concentrations are reported in Table 3 for tibia and vertebra and are illustrated in Fig. 2 for parietal bones.

Effect of growth. As for BMD measurements, Ca2+ and P concentrations increased between C0 and C28, more or less depending on the site considered, with the greatest differences observed in the parietal bone.

The Ca2+/P molar ratio was between 1.26 and 1.29 (except in tibial I SP, where it was 1.10) in the various

Table 1. Effects of spaceflight and recovery on bone length

<table>
<thead>
<tr>
<th>Groups</th>
<th>Femur</th>
<th>Tibia</th>
<th>Humerus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0</td>
<td>32.03 (31.53–32.13)*</td>
<td>36.78 (36.52–36.82)*</td>
<td>24.75 (24.50–24.99)*</td>
</tr>
<tr>
<td>F14</td>
<td>35.80 (35.45–36.52)</td>
<td>40.17 (39.65–40.46)</td>
<td>27.75 (27.11–27.66)</td>
</tr>
<tr>
<td>S14</td>
<td>35.22 (34.71–35.48)</td>
<td>39.60 (38.17–41.04)</td>
<td>27.26 (26.78–27.63)</td>
</tr>
<tr>
<td>S + R28</td>
<td>36.93 (36.60–37.23)‡</td>
<td>41.48 (40.65–41.72)‡</td>
<td>28.26 (28.24–28.79)‡</td>
</tr>
</tbody>
</table>

Values are medians (25th–75th percentiles) in mm. C0, control group killed on day of launch; F14, flight group killed at time of landing; S14, flight-synchronous group; F + R28, flight + recovery group; S + R28, flight-synchronous + recovery group; C28, control group killed at end of recovery period. Significantly different at P ≤ 0.05: *vs. C28, †vs. F14, ‡vs. S14.

Table 2. Effects of spaceflight and recovery on bone mineral density measured in the tibia and humerus

<table>
<thead>
<tr>
<th>Groups</th>
<th>Proximal</th>
<th>Mid</th>
<th>Entire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C0</td>
<td>160 (155–168)*</td>
<td>147 (141–150)*</td>
<td>151 (149–159)*</td>
</tr>
<tr>
<td>F14</td>
<td>151 (142–153)</td>
<td>157 (156–161)</td>
<td>158 (155–161)</td>
</tr>
<tr>
<td>S14</td>
<td>152 (141–160)</td>
<td>158 (156–167)</td>
<td>162 (157–165)</td>
</tr>
<tr>
<td>F + R28</td>
<td>164 (152–170)*</td>
<td>170 (161–172)*</td>
<td>163 (161–171)*</td>
</tr>
<tr>
<td>S + R28</td>
<td>161 (156–164)*</td>
<td>170 (169–178)*</td>
<td>169 (165–172)*</td>
</tr>
<tr>
<td>C28</td>
<td>186 (181–188)</td>
<td>183 (177–190)</td>
<td>181 (177–186)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Humerus</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C0</td>
<td>138 (135–141)*</td>
<td>146 (145–146)*</td>
<td>140 (139–141)*</td>
</tr>
<tr>
<td>F14</td>
<td>149 (139–150)</td>
<td>157 (155–163)</td>
<td>153 (152–157)</td>
</tr>
<tr>
<td>S14</td>
<td>149 (135–163)</td>
<td>159 (157–166)</td>
<td>157 (150–162)</td>
</tr>
<tr>
<td>F + R28</td>
<td>152 (149–176)*</td>
<td>171 (164–178)*</td>
<td>163 (159–172)*</td>
</tr>
<tr>
<td>S + R28</td>
<td>162 (153–164)*</td>
<td>166 (163–170)*</td>
<td>167 (163–170)*</td>
</tr>
<tr>
<td>C28</td>
<td>183 (179–188)</td>
<td>182 (175–183)</td>
<td>178 (174–180)</td>
</tr>
</tbody>
</table>

Values are medians (25th–75th percentiles) in mg/cm². Proximal subregions represent epiphysis, growth plate cartilage, and metaphysis. Mid subregions represent the diaphysis. Significantly different at P ≤ 0.05: *vs. C28, †vs. F14, ‡vs. S14 (only the more striking significative differences are reported).
Bone Histomorphometry

Effects of growth. The width of the I SP in the proximal metaphyses of the humerus (Table 4) and tibia (Fig. 3) was significantly decreased in older rats (C28), indicating that these animals were no longer in their rapid growth phase. Bone volume was also decreased in the tibia. This decrease was mainly associated with a decrease in trabecular thickness, while trabecular number remained unchanged (not shown). Endochondral bone growth was therefore reduced, leading to changes in I SP alone.

Effect of the 14-day spaceflight. Histomorphometric parameters measured in the great trochanter of the femur (Table 5) and the humerus did not differ between F14 and S14. Several events occurred in tibial metaphysis. I SP width and bone volume were reduced by 24 and 8%, respectively, in F14 (Fig. 3). In I SP, bone volume, TbTh, and TbN were decreased in F14 (Fig. 4) (48, 8, and 39%, respectively). The osteoid and osteoblastic surfaces of these areas were not significantly altered, although a trend toward a decrease (~10%) was observed in F14. In contrast, osteoclast surfaces were markedly increased by 91% in F14 (Fig. 4). Osteoclast number (cells/mm) was also higher in F14 than in S14 [22.4 (20.44–25.35) and 13.36 (13.19–15.21), respectively].

Bone sites of C0. This ratio was between 1.3 and 1.39 in C28 (except in tibial I SP, where it was 1.19).

Effect of the 14-day spaceflight. No statistical difference in Ca$^{2+}$ and P concentrations or Ca$^{2+}$/P molar ratio was observed in the tibial cortices between F14 and S14. In the tibial I SP, the Ca$^{2+}$ and P contents were slightly but significantly lower in F14 than in S14. In the same site, the Ca$^{2+}$/P molar ratio was also lower in F14 than in S14 (1.07 vs. 1.15). In the tibial II SP of F14, Ca$^{2+}$ and P concentrations were both lower than those of S14 (approximately 13% for Ca$^{2+}$ and P). The Ca$^{2+}$/P molar ratio decreased to a value close to 1.09 in F14 compared with 1.15 in S14.

The Ca$^{2+}$ and P concentrations and Ca$^{2+}$/P molar ratio were not modified in the cortices of vertebral bodies of F14 and S14. In vertebral I SP, calcium was significantly increased by 2.7% in F14 compared with S14. The Ca$^{2+}$/P molar ratio was higher in F14 (1.4) than in S14 and C0 (1.27 and 1.28, respectively). Finally, the Ca$^{2+}$ and P concentrations in parietal bone were significantly increased by 4 and 8%, respectively, in F14 compared with S14. The Ca$^{2+}$/P molar ratio was significantly increased in F14 (1.39) compared with S14 and C0 (1.28 and 1.27, respectively).

Effects of a 14-day reambulation period after the 14-day spaceflight. In tibial cortices, no statistical change in Ca$^{2+}$ and P concentrations and Ca$^{2+}$/P molar ratio was observed between the F + R28 group and its synchronous group S + R28. In I SP, calcium content was still decreased by 3.8% in F + R28 compared with S + R28. No difference was observed in the Ca$^{2+}$/P molar ratio. In II SP, Ca$^{2+}$ and P contents were decreased by 1.6 and 0.5%, respectively, in F + R28 compared with S + R28. No statistical variation was observed between C28 and S + R28. The Ca$^{2+}$/P molar ratio still remained lower in F + R28 than in S + R28 (1.27 and 1.36, respectively).

No difference was observed in vertebral bodies between the F + R28, S + R28, and C28 groups.

In parietal bones, the Ca$^{2+}$ concentration was still increased by 1.6% and the P concentration was still increased by 2% in F + R28 compared with S + R28. Ca$^{2+}$ and P concentrations in C28 and S + R28 groups were not significantly different. The Ca$^{2+}$/P molar ratio was significantly higher in F + R28 than in S + R28 (1.39 vs. 1.30).

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No difference was observed in vertebral bodies between the F + R28, S + R28, and C28 groups.

In parietal bones, the Ca$^{2+}$ concentration was still increased by 1.6% and the P concentration was still increased by 2% in F + R28 compared with S + R28. Ca$^{2+}$ and P concentrations in C28 and S + R28 groups were not significantly different. The Ca$^{2+}$/P molar ratio was significantly higher in F + R28 than in S + R28 (1.39 vs. 1.30).
Effects of a 14-day reambulation period after the 14-day spaceflight: comparison between F + R28 and F14; S + R28 and S14; and F + R28, S + R28, and C28. Flight and synchronous rats both exhibited more alterations after the reambulation period than after the flight period, as the femoral trochanter area, which was not modified after flight, showed decreased osteoblastic surfaces in F + R28 compared with F14 (30%), suggesting early signs of bone formation impairment. The trabecular number was also decreased in S + R28 compared with S14 (24%), suggesting that bone resorption had been previously and transiently increased.

In the humerus, I SP width was lower in S + R28 than in F + R28 (−32%), S14 (−9%), and C28 (−21%), indicating that synchronous animal growth was affected after the space simulation period. Despite the reduced area occupied by the humeral I SP, the percentage of bone volume was not modified in any of the groups. Bone loss occurred in the humeral II SP of F + R28 compared with F14 (−30%), S + R28 (−37%), and C28 (−40%) and was related to loss of complete trabeculae with no concomitant decrease in thickness of the remaining trabeculae. At the cellular level, osteoblastic and osteoclastic parameters were not significantly different between the groups. Humeral bone loss in II SP, which was only slight (and not significant) at the end of the flight, was accentuated 14 days later.

In the tibia (Fig. 3), I SP was thinner in S + R28 than in F + R28, S14, and C28. Primary SP bone volume was not affected in F + R28 compared with C28. An active recovery phase seemed to occur in synchronous rats, since S + R28 bone volume and TbTh were greater than in C28 and F + R28. In II SP (Fig. 4), bone loss worsened in F + R28 compared with F14 (by 42%), due to a decreased trabecular number (56%). Bone mass and structural parameters of S + R28 were not different from those of S14 or from those of C28. At the cellular level, a very active recovery phase with a rebound of bone formation was observed in F + R28 versus F14 (57% for osteoid surface/bone surface and 69% for osteoblastic surface/bone surface). An increase in resorption activity at the end of the reambulation period was visible in F + R28 compared with both F14 and S + R28 (although to a lesser extent compared with that seen immediately after the flight between F14 and S14).

All results (including densitometry, histomorphometry, and X-ray microanalysis) are summarized in Table 6.

**DISCUSSION**

The present study investigated the effects of a 2-wk spaceflight followed by a 2-wk reambulation period on earth on the bones of young male rats. This experiment showed various alterations, with differences between different bone sites and within the same bone.

**Effects of Spaceflight and Immediate Return**

After the flight, densitometric measurements did not reveal any change in the tibia and humerus. A decrease in femoral BMD was demonstrated in flight rats com-
Bone, Spaceflight, and Recovery

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Fig. 2. Box (25th-50th percentile) and whisker (10th and 90th percentiles) plots of Ca²⁺ and P concentrations measured in parietal bones. Open boxes, control rats; shaded boxes, spaceflight rats; hatched boxes, synchronous rats. Only the most striking significant differences (P < 0.05) are reported.

Compared with their synchronous counterparts. The sensitive region of the femur was the distal end, containing a large metaphysis, whereas the diaphysis and proximal end were not altered. The BMD value of distal femur was similar in flight and basal control rats, suggesting that bone growth was inhibited by spaceflight conditions. Control animals killed on day 0 (C0) and day 28 (C28) continued to accumulate minerals: markedly in the femur, to a lesser extent in the humerus, and to an even lesser degree in the tibia. Furthermore, the BMD increase was lower in the proximal part of the tibia than in the diaphysis, whereas an opposite effect was observed in the humerus. The femur, which seemed to accumulate the greatest amount of minerals, suggesting that it was the least mature bone, therefore appeared to be the bone most sensitive to microgravity or affected the earliest. In bone samples submitted to X-ray microanalysis, i.e., parietal bone, thoracic vertebra, and proximal tibia, a site-dependent increase in both Ca²⁺/P molar ratio and Ca²⁺ and P concentrations was also observed in C28 versus C0. The Ca²⁺/P molar ratio was most markedly increased in both the cortical and trabecular compartments of the tibia, while the greatest increase in Ca²⁺ and P concentrations was observed in the parietal bones. In proximal tibia, exposure to space conditions decreased Ca²⁺ and P concentrations and the Ca²⁺/P molar ratio only in cancellous bone. The Ca²⁺/P molar ratio was ~1.09 in tibial II SP of F14 (compared with 1.25 in S14), which is close to the brushite Ca²⁺/P molar ratio (1.03) (11). Brushite has been proposed to be one of the precursors of hydroxyapatite (7). These data suggested that mineral maturation was inhibited or delayed after flight in cancellous bone of the tibial metaphysis.

The contents of various organic and inorganic compounds have been previously measured in space-flown rats (5, 12, 20). In general, the results have indicated impaired organic matrix and/or mineralization pattern leading to reduced biomechanical properties. For example, a marked reduction in the fraction of bone minerals was reported for the whole body after the 21-day Cosmos 1129 flight (17). During the same mission, calcium content was lower in the vertebral centrum of flight rats, whereas PO₄³⁻ remained unchanged, suggesting incomplete osteoid mineralization (19). After the 7-day Spacelab 3 mission, Patterson-Buckendahl et al. (16) observed a decrease in total vertebral and humeral bone mass and calcium and inorganic phosphorus contents in flight rats versus synchronous controls and Simmons et al. (21) reported an alteration of femoral hydroxyapatite maturation. Mineralization impairment therefore occurred in both weight- and non-weight-bearing bones by the first week of microgravity exposure. After the 12.5-day Cosmos 1887 flight, followed by 2.5-day recovery, Mechanic et al. (12) reported a significant reduction in bone mineral content in the femoral diaphysis, whereas no such changes were identified in the humeral diaphysis after the 12.5-day Cosmos 2044 flight, which did not include recovery (25). Our data are in line with these last results. A close correlation has been previously demonstrated between BMD and Ca²⁺ and P contents evaluated by electron probe analysis and chemical analysis of Ca²⁺ or P contents (4, 28). The densitometric and electron probe results obtained in the present study suggested that hindlimb cortexes were not affected after a 2-wk microgravity exposure. Similarly, Kaplan-Sky et al. (10) found no change in tibial compact bone after a 2-wk Cosmos flight, whereas the cancellous envelope appeared to be altered. X-ray microanalysis and histomorphometry revealed changes in the tibial proximal metaphysis not detected by densitometric measurements. Because the epiphysis and cortical bones remained unchanged, it is possible that the bone alterations in the cancellous bone of the proximal tibial metaphysis may not have been sufficiently marked to be detected by overall densitometric measurement of the proximal part of the tibia.

X-ray analysis showed a slight but significant increase in Ca²⁺ and P concentrations and Ca²⁺/P molar ratio in vertebral II SP and more marked increases in the parietal bones of F14 versus S14. Mineralization profiles performed after the 12.5-day Cosmos 1887 flight showed opposite results in calvaria (20). These discrepancies are difficult to explain, but could be related to the 2.5 days spent under stressful conditions after landing of Cosmos 1887. Our results suggested an
acceleration of bone mineral maturation in the skull after a 2-wk spaceflight. Such findings have already been observed in rats after 3 wk of tail suspension (18). A biochemical study conducted by Arnaud et al. (2) showed that the skull and jaw calcium content of rats was increased by 15% after a 2-wk tail suspension. The present study is the first to report such results in rats after a 2-wk spaceflight. Such findings have already been observed in rats after 3 wk of tail suspension (18). As seen after the 2-wk Cosmos 2044 flight (26) involving 300-g Wistar male rats, the present flight induced a decrease in tibial elongation rate. In contrast, whereas only a trend toward bone cellular decoupling was observed after the Cosmos 2044 flight, an increased bone resorption activity and a more dramatic bone loss in II SP was observed in the present study. Because both American and Russian 2-wk space missions only showed nonsignificant trends toward a decrease in bone formation in tibial II SP, no definitive conclusions can yet be drawn, but two hypotheses can be formulated.

After 2 wk, bone formation might return to normal following the decrease that we observed after a 1-wk flight (29). This would be in line with the results reported in the rat tail-suspension model (27). However, this comparison concerns another species of heavier rats.

The reduction of bone formation may have started, and a significant change can only be observed after more than 2 wk. In the 7-day SLS1 mission, rats only exhibited a trend toward decreased bone formation...
However, although a similar strain and sex of rats was used in the SLS1 and SLS2 missions, SLS2 rats were much younger than SLS1 rats. This brief review of literature emphasizes the difficulty of comparisons between missions due to differences in experimental animals. In-flight tetracycline labeling would be particularly useful to evaluate the dynamics of osteoblastic activity. Such labeling was performed in Sprague-Dawley rats during the 10-day American PSE-2 mission. The bone formation rate was decreased in the cancellous compartment of the proximal humeral metaphysis (3). However, these rats were younger than our rats, so these events might have occurred earlier.

In the present study, bone volume was unaffected in the proximal humerus, probably due to a lower modeling/remodeling rate.

Effects of a 2-wk Reambulation Period

At the end of the spaceflight, the BMD was lower in F14 than in S14 in only a few femoral sites. Conversely, the BMD in all sites except for the humeral diaphysis was decreased after reambulation in F + R28 compared with C28. The harmful effects of flight were therefore delayed. In some zones, mainly trabecular zones such as the proximal tibia and humerus, growth was even arrested, as no difference was observed between rats in the F14 and F + R28 groups. Simulation also induced bone loss, as all BMD values (except in the femoral diaphysis) were lower in S + R28 than in C28. In summary, the effects of spaceflight and simulation were not observed immediately after return, but later, after the reambulation period. The decrease in bone mass after reambulation could also be related to weight loss of the animals, as observed in rats killed 6 days after landing of the Cosmos 1129 flight (8). Landing per se may also have influenced bone cell activities.

Interestingly, the decrease in mineral concentrations, measured by X-ray microanalysis, observed after the flight did not worsen after the reambulation period. In the cancellous part of the proximal tibial metaphysis, and in the vertebral II SP and parietal bone, trends toward normalization of mineralization were observed, indicating the existence of an active, although incomplete, recovery process. It is noteworthy that Ca$^{2+}$ and P concentrations and the Ca$^{2+}$/P molar ratios were similar in S + R28 and C28. This analysis therefore demonstrated a specific effect of spaceflight on bone minerals, independent of stress-related conditions.

Histomorphometric analysis confirmed the harmful effect of spaceflight on bone mass demonstrated by densitometry and the active recovery process on bone formation parameters suggested by the microanalysis data. It also defined various types of events.

Table 5. Effects of spaceflight and recovery on histomorphometric parameters measured in the femoral fossa trochanteri

<table>
<thead>
<tr>
<th>Groups</th>
<th>BV/TV, %</th>
<th>TbTh, µm</th>
<th>TbN, no./µm</th>
<th>OS/BS, %</th>
<th>ObS/BS, %</th>
<th>Oc/BPm, cells/µm</th>
<th>OcS/BS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0</td>
<td>26.91</td>
<td>58</td>
<td>4.38</td>
<td>10.22</td>
<td>19.6</td>
<td>4.77</td>
<td>5.63</td>
</tr>
<tr>
<td>F14</td>
<td>22.5</td>
<td>61</td>
<td>3.7</td>
<td>10.73</td>
<td>12.96</td>
<td>5.28</td>
<td>5.32</td>
</tr>
<tr>
<td>S14</td>
<td>26.51</td>
<td>65</td>
<td>3.83</td>
<td>9.06</td>
<td>15.21</td>
<td>5.57</td>
<td>6.74</td>
</tr>
<tr>
<td>F + R28</td>
<td>27.99</td>
<td>71</td>
<td>3.63</td>
<td>11.48</td>
<td>9.18t</td>
<td>5.82</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td>(23.18–29.04)</td>
<td>(65.75–78.00)</td>
<td>(3.26–4.12)</td>
<td>(7.56–13.19)</td>
<td>(8.29–11.18)</td>
<td>(4.09–6.23)</td>
<td>(5.29–6.82)</td>
</tr>
<tr>
<td>S + R28</td>
<td>20.02</td>
<td>69</td>
<td>2.92t</td>
<td>11.34</td>
<td>16.23</td>
<td>6.59</td>
<td>6.81</td>
</tr>
<tr>
<td></td>
<td>(17.28–23.45)</td>
<td>(60.00–73.25)</td>
<td>(2.86–3.21)</td>
<td>(10.3–14.64)</td>
<td>(10.81–16.80)</td>
<td>(5.46–6.32)</td>
<td>(6.08–7.32)</td>
</tr>
<tr>
<td>C28</td>
<td>24.4</td>
<td>70</td>
<td>3.66</td>
<td>12.39</td>
<td>11.62</td>
<td>4.95</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>(22.80–27.29)</td>
<td>(63.00–77.25)</td>
<td>(3.04–4.09)</td>
<td>(7.23–14.64)</td>
<td>(10.92–19.00)</td>
<td>(4.72–5.77)</td>
<td>(5.69–7.25)</td>
</tr>
</tbody>
</table>

Values are medians (25th–75th percentiles). Significantly different at P ≤ 0.05: † vs. F14, ‡ vs. S14.
The effects of simulation could not be precisely evaluated due to the absence of a vivarium control group on day 14. On day 28, an active recovery process was observed in I SP bone mass parameters of S1R28, as I SP tibial bone volume and TbTh were higher in S + R28 than in C28, whereas no difference was observed in the humerus. No other effect of simulation was observed in the other bone sites. F1R28 also exhibited a decrease in tibial longitudinal growth rate compared with C28, whereas bone mass and microarchitectural parameters were similar between these two groups.

In view of the data reported in the literature, we expected to find an incomplete recovery in tibial II SP (8, 31). In our study, accentuation of tibial bone loss was related to loss of trabeculae (42% between F14 and S14; 37% between F + R28 and F14). Bone loss was only observed after the reambulation period in the humerus (30% and 36% between F + R28 and F14 for bone volume/total volume and TbN, respectively). In both humerus and tibia, the dramatic reduction of TbN with no reduction of TbTh suggested an increase in resorption activity. We did not observe any such increase in osteoclastic parameters in the humeral II SP and only a slight increase was observed in the tibia, less marked than that observed immediately after the flight. The tibial bone formation parameters, unchanged after the flight, were dramatically increased after reambulation and reached higher levels than in controls. In the tibia, despite the bone loss observed in the II SP, the active recovery process, including intense bone formation and normal resorption, could possibly lead to a positive bone balance. Successful recovery of periosteal bone formation was reported 26 days after the 19.5-day Cosmos 782 flight (19). After the Cosmos 1129 flight, the results were indicative of delayed maturation of bone mineral and matrix. A return to normal was observed on the 29th day postflight (22). Our results showed that the time required to recover bone mass was longer than the flight duration. On earth, young rats unloaded for a period of 2 wk, then reloaded for up to 2 wk (19), showed an increased bone formation rate at the tibiofibular junction and a 30–40% increase in tibial and lumbar vertebral calcium accretion after reloading, compared with normally loaded rats. We showed that the tibia of old rats suspended for 14 days and reloaded for 28 days did not recover in terms of bone mineral content and BMD (28). These studies showed that the quantity of bone lost in space or on earth was not entirely recovered, but that osteoblastic function and mineral accretion (both assessed by histomorphometry and electron analysis) seemed to recover and even showed a rebound. The reason for this discrepancy is not yet clear. Sequential measurements, not
only of bone formation but also of bone resorption, which would be helpful. In particular, it would be interesting to determine whether the regional acceleratory phenomenon (6) that follows stressful situations, such as landing, is responsible for bone loss.

In the femoral fossa trochanter, facing Sharpey’s fibers in which no change was observed after the flight as previously reported (26), a decrease in osteoblastic fibers in which no change was observed after the flight and an increase in resorption and delayed bone formation occurred, thus emphasizing the need for long-term follow-up of astronaut skeletons.

**Conclusion**

In the present study, we described the effects of a 14-day spaceflight in various bone sites: early and major changes in the tibia, less marked and delayed changes in the humerus, and minor changes in the greater trochanter of the femur. The order and magnitude of these events confirmed the observations that we reported after the Biocosmos flights. X-ray microanalysis showed for the first time that bone-crystal maturity increased in vertebral II SP, and decreased in tibial II SP. In terms of bone cell kinetics, we confirmed the increase in bone resorption after the second week of spaceflight. However, histodynamic studies are necessary to determine whether bone formation is significantly decreased or normalized, as observed in the rat tail suspension model (27). Postflight data showed that bone loss either occurred or was accentuated after a reambulation period equal to the duration of the flight, whereas mineralization and bone formation parameters indicated the onset of an active recovery process. This study led to a better understanding of the bone response to space because of the use of three different techniques (histomorphometry, X-ray microanalysis, and double X-ray densitometry), which gave complementary information at the tissue and cellular levels.

**Perspectives**

Spaceflight represents the ultimate challenge that allows a better understanding of the role of gravity in physiology. The mechanical unloading provided by microgravity exposure has pronounced but not well-known effects on bone tissue. Histomorphometric studies of bones from rats after space missions of various periods provided the time course of the cancellous bone cellular events; transient increase in resorption and sustained decrease in bone formation. In the present study we report the effects of a 14-day space flight on young rats, followed by a 14-day recovery period. We show that the flight-induced bone mass changes do not affect skeleton homogeneously, thus emphasizing the
need to perform measurements at different levels: weight- and non-weight-bearing bones and cortical and trabecular compartments. Local concentrations of Ca\(^{2+}\) and P (evaluated by X-ray microanalysis) increased in the skull and decreased in tibia, suggesting that minerals might have been redistributed. After 14 days of reambulation, the bone loss worsened; however, a dramatic increase in bone formation indicated that an active recovery process had taken place. Thus the postflight period deserves more attention than it is currently receiving. Further experiments should take the following guidelines into account: use of adult rats given the influence of growth in the young animals, fluorochrome labelings during the mission for studying dynamic formation activity, and in-flight killing to avoid the landing effects.

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Address for reprint requests: M.-H. Lafage-Proust, Laboratoire de Biologie du Tissu Osseux, GIP “Exercice,” Faculté de Médecine, 15 rue Ambroise Pare, 42023 Saint-Etienne, France.

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