Two-week longitudinal survey of bone architecture alteration in the hindlimb-unloaded rat model of bone loss: sex differences

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David, Valentin, Marie-Hélène Lafage-Proust, Norbert Laroche, Alexandre Christian, Peter Ruegsegger, and Laurence Vico. Two-week longitudinal survey of bone architecture alteration in the hindlimb-unloaded rat model of bone loss: sex differences. Am J Physiol Endocrinol Metab 290: E440–E447, 2006; doi:10.1152/ajpendo.00293.2004.—The goal of this study was to determine, through a longitudinal follow-up, whether sex influences bone adaptation during simulated weightlessness. Twelve-week-old male and female Wistar rats were hindlimb unweighted for 2 wk, and the time course of bone alteration was monitored in vivo by means of densitometry and unbiased three-dimensional quantitative microcomputed tomography at 7 and 14 days. Compared with male rats, female rats had twice more cancellous bone volume at the proximal tibia at baseline, and this bone volume continued to increase, whereas in males it stabilized. Conversely, cortical area was greater in males than in females, and in both sexes cortical bone was still expanding. Hindlimb unloading resulted in larger reductions in males than in females in both cortical and cancellous compartments. In females, trabecular thickness and number decreased mildly, whereas in males trabecular number was dramatically reduced. In both sexes, the trabecular network became less connected and more rod-like shaped. Bone cellular activities evaluated by histomorphometry showed decreased bone formation rate in both sexes and increased resorption activity only in males. In conclusion, in female rats unloading resulted in larger reductions in males than in females, and in both sexes cortical and cancellous compartments that allowed comparing not only end points but also kinetics of bone changes during the experimental period. To achieve this goal, we used a novel microcomputed tomograph (μCT) designed to noninvasively monitor bone microarchitecture in living rodents (7).

MATERIALS AND METHODS

Animals

The protocol and animal procedure for this study were in compliance with the European Community standards on the care and use of laboratory animals (Ministère de l’Agriculture, France, Authorization no. 04827). Fourteen male (265 ± 15 g) and 14 virgin female (250 ± 10 g) Wistar-Han rats (Charles River Laboratories, l’Arbresle, France), 12 wk old, were randomly assigned to two groups of 14 animals each. Male and female groups were further divided into two subgroups: tail suspended (S) or maintained in suspension cages but not suspended (control groups, Ctr), for 14 days. The rats were acclimatized for 1 wk with standard temperature (23 ± 1°C) and 12:12-h light-dark conditions. Animals were individually housed and provided with food (standard diet) and water ad libitum. The suspension procedure was performed according to the Globus et al. (11) recommendations. Fluorochrome double bone labeling was performed 4 days and 1 day before animals were killed. The rats received an intraperitoneal injection of 30 mg/kg tetracycline (Sigma Aldrich).

μCT and dual-energy X-ray absorptiometry (DEXA) measurements were performed on days 0, 7, and 14. Before measurement, rats were anesthetized by an intraperitoneal dose of 100 mg/kg ketamine (Panpharma, France) and 8 mg/kg xylazine (Rompun, Bayer Pharma, weightlessness model compared with females. The gold standard bone loss model in females is ovariectomy-induced bone loss, and it was proposed that postmenopausal osteoporosis per se would be a failure of the bone’s adaptation to functional loading (22). However, in rats, it has been recently shown that estrogens suppress the responsiveness of the female skeleton to mechanical loading (19). Sex-specific response during immobilization has been poorly investigated. To our knowledge, this question has been addressed in only one study in the Fischer 344 inbred strain of rats (13). Using histomorphometry at the proximal tibia, Hefferan et al. (13) found that the bone loss in hindlimb-unweighted male and female rats appears to be due to similar mechanisms. To further elucidate the sex-specific response to immobilization, we studied the Wistar outbred strain of rat during a 2-wk experiment using the tail suspension model. We performed a longitudinal survey of both cancellous and cortical compartments that allowed comparing not only end points but also kinetics of bone changes during the experimental period. To achieve this goal, we used a novel microcomputed tomograph (μCT) designed to noninvasively monitor bone microarchitecture in living rodents (7).

GROWTH AND DEVELOPMENT OF RATS encompasses a broad range of research including the bone field. Rodent partial immobilization by tail suspension, originally a model of simulated hypogravity, is now one of the most used for inducing regional bone loss. It has been extensively used in parallel to the Russian unmanned Biocosmos flight series comprising male Wistar rats (24, 25). These studies showed ample evidence of pathophysiological functional and structural alterations in muscle, bone, cardiovascular, and related body fluid shift responses. In the skeleton, unloading induced both cortical and cancellous bone loss in the hindlimbs (11, 29). Longitudinal elongation rate was found either diminished or unchanged (8, 27). However, as male rats were more often used in space missions, they were also more often involved in this simulated...
France), and the animals were killed with a high dose of Nesdonal (Specia, Paris, France).

The left tibia of each animal was measured in vivo and ex vivo by µCT and DEXA, respectively, and bone sections from the same sample were then processed for conventional histomorphometry.

**DEXA**

A dual-energy X-ray PIXImus densitometer (LUNAR, Madison, WI) with small-animal software was used for measuring bone mineral density (BMD) and bone mineral content (BMC). It is a rapid (5-min image acquisition) and precise small-animal densitometer (4), with a precision of 1% coefficient of variation (CV) for total skeletal BMD, and 1.5% CV for femoral BMD. After the animal measurement was completed, the region of interest (ROI) rectangle was moved and reshaped to cover a portion: entire left tibias (ET), left femora (EF), and left humeri (EH) were analyzed. The PIXI software automatically calculated bone density and recorded the data as Microsoft Excel files.

**In Vivo High Resolution µCT**

The left tibiae were scanned with a high-resolution µCT prototype (VivaCT20) from Scanco Medical (Scanco Medical, Bassersdorf, Switzerland), recently described by David et al. (7). This apparatus performs noninvasive in vivo examination of bones of small laboratory animals with a high resolution. Voxel matrix was 20 × 20 × 26 µm³. The scanned region corresponded to a zone of 253 transverse slices (6.58 mm) under the left proximal growth plate of tibia. The net scanning time was ~10 min. From the acquired data, the ROI in the axial direction was delimited anatomically from the bottom of the primary spongiosa (7). The height of the ROI was delimited with regard to cancellous bone distribution in the tibial metaphysis. Because there is a larger amount of cancellous tissue in females' metaphysis than in males' (Fig. 1A), the ROI was delimited up to a height of 2.6 mm (100 slices) for female animals and 1.3 mm (50 slices) for males in order to analyze the whole secondary spongiosa. Then we manually adjusted the ROI top at the primary-secondary spongiosa boundary susceptible to varying according to growth or bone loss processes. For each transverse slice, the ROI was established to encompass as much as possible of cancellous bone. All gray-scale images were segmented using a Gaussian filter and a fixed threshold (15% of maximal gray-scale, corresponding to a value of 150) for all data (7).

**Three-Dimensional Trabecular Parameters**

The bone volume fraction was calculated directly by plotting gray voxels representing bone fraction against gray plus black voxels (nonbone objects) (VOX BV/TV). Bone surface (BS) was calculated using a tetrahedron meshing technique generated by the “marching cubes method” (23), and total volume (TV) was taken as the volume of interest (VOI). The normalized indexes (BV/TV, BS/TV, and BS/BV) were used.

Three-dimensional (3-D) metric indexes were calculated using direct techniques based on the distance transformation (15, 16), without assuming a constant model. Direct indexes were calculated as follows:

- Tb.Th was calculated by filling maximal spheres into a structure, and the average thickness of all voxels corresponded to Tb.Th.
- The same procedure was used to determine Tb.Sp, but in this case the nonbone voxels were filled with maximal spheres, and the mean thickness of the marrow cavities represented Tb.Sp.
- Tb.N was the inverse of the mean distance between the midaxes of the observed structure. The midaxes of the structure were assessed from the binary 3-D image using the 3-D distance transformation and by extracting the center points of nonredundant spheres that fill the structure completely. The mean distance between the midaxes was then determined by analogy with the Tb.Sp calculation.
- The plate-rod characteristic of the structure was estimated by the structure model index (SMI) (16) calculated by differential analysis of a triangulated surface of a structure: SMI = 6{[BV(dBS/dr)]/BS²}, where dBS/dr is the surface area derivative with respect to a linear r (half-thickness or the radius assumed to be constant over the entire structure). The SMI value is 0 for an ideal plate, and 3 for an ideal rod structure. Values between 0 and 3 correspond to a structure with both plates and rods, depending on the volume ratio between rods and plates.

The geometric degree of anisotropy (DA) is defined as the ratio between the maximal and minimal radius of the mean intercept length ellipsoid (12, 30).

Connectivity density (Conn.Den.) was calculated using the Euler method of Odgaard and Gundersen (26).

**Cortical Measurement**

To analyze the cortex, we chose a cross-sectional slice among the original images on which the distance between the tibia and fibula was ~4 mm. We assumed that the individual distance was constant, and for each measurement point, cortical area (Cl.Ar.), cross-sectional or total area (T.Ar.), and marrow Area (Ma.Ar.) were evaluated, with the same Gaussian filter and the same fixed threshold as for the trabecular structure (Fig. 1B).

**Histomorphometric Analysis**

The proximal tibial metaphyses were fixed in 4% formaldehyde solution, dehydrated in acetone, and embedded in methylmethacrylate (6). Longitudinal frontal slices were cut from the embedded bones with a Jung model K microtome (Carl Zeiss, Heidelberg, Germany). Six nonserial sections, 8 µm thick, were used for modified Goldner staining (3). Fourteen-micrometer-thick sections were used to determine the dynamic indexes of bone formation (dLS/BS, MAR, BFR/BS). MAR was derived from fluorochrome interlabel distances. BFR were subsequently obtained from the product of dLS/BS and MAR (7). Six-micrometer-thick sections were used for tartrate-resistant acid phosphatase (TRACP) staining (6, 7), allowing determination of osteoclastic parameters (Oc.S/BS, N.Oc/B.Ar). Histological parameters were evaluated on a region matching with 3-D µCT ROI (Fig. 1A). Bone volume and parameters reflecting trabecular structure were measured using an automatic image analysis system (Biocom, Lyon, France). Bone cellular and macroscopic parameters were measured with a semiautomatic system: digitizing tablet (Summasketch; Summagraphics, Paris, France) connected to a Macintosh personal computer with software designed in our laboratory (3).

**Statistical Analysis**

The statistical test analysis was performed using commercially available statistical software (Statistica; StatSoft, Tulsa, OK). One-way ANOVA (sex factor) was performed between baseline values for densitometric and tomographic data. A three-way ANOVA was performed, with two between-group factors (male or female, tail-suspended or control) and a repeated-measures factor (within subjects factor). In parallel, we compared the time kinetics of different groups by comparing slopes of individual linear regression curves. The individual slopes were compared using a two-way ANOVA with two between-group factors (male or female, tail-suspended or control). When F values for a given variable were found to be significant, the sequentially rejecting Bonferroni-Holm test (17) was subsequently performed using the Holm’s adjusted P values taken from the t-table. Results were considered to be significantly different at P < 0.05.

Two-way analysis of variance (ANOVA) was performed on histomorphometric data to determine the influence of both suspension and
sex factors on the structural and cellular parameters. When $F$ values for a given variable were found to be significant, a post hoc Scheffé test was performed, and the results were considered to be significantly different at $P < 0.05$.

RESULTS

Differences Between Males and Females in Controls

Body weight. Male rats were 1.5-fold heavier than females despite their identical age ($P < 0.00001$). During the experimental period, male rats increased their body weight threefold more than female rats ($P < 0.036$; Fig. 2).

DEXA assessment of bone alteration. At baseline, femoral BMD was similar in both sexes (Table 1). Baseline tibia BMD was significantly lower in male compared with female rats ($P < 0.034$). No difference was observed in the humerus between sexes (not shown).

Microarchitecture of tibial metaphysis. Baseline values of BV/TV and Tb.N were 58 and 50%, respectively, lower in males than in females ($P < 0.0004$). During the experiment, BV/TV plateaued in males and significantly increased with time in females (mean slopes: 0.17 vs. −0.09, $P < 0.05$; Fig. 3A). Tb.N slowly declined with aging in males (mean slope = −0.026 trabeculae·mm$^{-1}$·day$^{-1}$), whereas it increased by 0.011·mm$^{-1}$·day$^{-1}$ ($P < 0.05$ vs. male Ctr) in females (Fig. 3B). Baseline Conn.Den. values were nearly threefold lower for males than for females ($P < 0.03$), and kinetics throughout the experiment paralleled those observed for Tb.N (Fig. 3C). Trabeculae in males were 20 µm thinner than in females. Evolution of Tb.Th looked similar between male and female groups (Fig. 3D). In males, SMI was indicative of a rod-like structure, whereas females had an intermediate rod-plate trabecular structure (3.2 vs. 2, $P < 0.02$; Fig. 4A). During the

Fig. 1. Microtomographic reconstructions of 26-µm-thick bone slices. A: frontal slice of proximal tibia of a male (left) and female (right). Limits of region of interest (ROI) are delineated by dotted line. B: transversal section of tibial diaphysis in male (left) and female (right).
experiment, the pattern evolved toward a more plate-like model in both sexes. Sex greatly influenced the DA age-related kinetics; males presented a positive slope, females a negative one (Fig. 4B). Males had an ~20% greater Ct.Ar than females (Figs. 1B and 5) at baseline. During growth, cortex continued to enlarge in a similar way in males and females.

**Bone cellular activities.** Parameters of bone cellular activities, evaluated at the secondary spongiosa level, are presented in Table 2. MAR was 24% lower in males than in females, whereas dLS/BS was similar in both sexes. Active resorption surfaces were 93% more important in males than in females.

**Male Suspension vs. Male Controls**

**Body weight.** Suspension did not induce a decrease in body weight in males, but only a smaller increase than in Ctr group ($P < 0.013$; Fig. 2).

**DEXA assessment of bone alteration.** In suspended rats, femoral BMD values slowly decreased with time ($-0.003 \pm 0.03$ g/cm$^2$·day$^{-1}$) compared with the Ctr group, whereas no change in tibia BMD was noted (Table 1). Suspension did not alter humeral growth (not shown).

**Microarchitecture of tibial metaphysis.** Suspension induced bone loss (BV/TV, 54% during the experiment, mean slope: $-0.41, P < 0.012$; Fig. 3A) and accentuated the Tb.N (33% loss during suspension, mean slope: $-0.66, P < 0.05$) and Conn.Den. (26% loss during suspension, mean slope: $-0.95, P < 0.05$) age-related decline (Fig. 3, B and C). Tb.Th was not significantly affected; also, the slope was descending (Fig. 3D). The SMI pattern evolved toward a more rod-like structure in the S groups throughout the experimental period ($P < 0.02$ vs. Ctr) (Fig. 4A). During the experiment, a progressive DA increase appeared more important during suspension than in Ctr rats (11%, $P < 0.05$; Fig. 4B). Ct.Ar decreased by 12% ($P < 0.05$) during the experiment in suspended rats (Fig. 5).

**Bone cellular activities.** Suspension induced a significant decrease in dLS/BS (50%, $P < 0.02$). BFR/BS alteration paralleled that of dLS/BS. N.Oc./B.Ar increased significantly after 14 days in the S group compared with Ctr (40%, $P < 0.0001$; Table 2).

**Female Suspension vs. Female Controls**

**Body weight.** Despite the lack of statistical difference between female S and Ctr rats’ body weight evolution, a slow decrease was noted in the female S group (mean slope = $-0.18$ g/day; Fig. 2).

**DEXA assessment of bone alteration.** In females, femoral and tibia BMDs were not altered by suspension, as they increased at a similar rate in both S and Ctr rats (Table 1). Suspension did not alter humeral growth (not shown).

**Microarchitecture of tibial metaphysis.** Suspension induced a 15% decrease in BV/TV (mean slope: $-0.21, P < 0.0007$; Fig. 3A), a 2.3% decrease in Tb.N (mean slope: $-0.02, P < 0.05$; Fig. 3B), a 22% decrease in Conn.Den. (mean slope: $-0.78, P < 0.05$; Fig. 3C), and a 5% decrease in Tb.Th (mean slope: $-0.0003, P < 0.05$; Fig. 3D). The SMI pattern evolved toward a more rod-like structure in the S groups throughout the experimental period ($P < 0.02$ vs. Ctr). Suspension had no major effect on the DA age-related kinetics, although the slope appeared less negative than in Ctr rats (Fig. 4B). Suspension decreased Ct.Ar by 6%.

**Bone cellular activities.** Suspension induced a significant decrease in dLS/BS (48%, $P < 0.02$). BFR/BS alteration

### Table 1. Suspension- and aging-induced changes in bone densitometric parameters of the entire left femora and left tibiae

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Femoral BMD ($g/cm^2$)</th>
<th>Tibial BMD ($g/cm^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Ctr</td>
<td>Baseline 0.202±0.014</td>
<td>0.130±0.015</td>
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<tr>
<td></td>
<td></td>
<td>7 days 0.223±0.007</td>
<td>0.133±0.007</td>
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<tr>
<td></td>
<td></td>
<td>14 days 0.224±0.007</td>
<td>0.141±0.005</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Baseline 0.215±0.013</td>
<td>0.136±0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days 0.215±0.014</td>
<td>0.134±0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 days 0.210±0.013*</td>
<td>0.136±0.010</td>
</tr>
<tr>
<td>Females</td>
<td>Ctr</td>
<td>Baseline 0.217±0.013</td>
<td>0.147±0.008*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days 0.220±0.011</td>
<td>0.146±0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 days 0.228±0.010</td>
<td>0.152±0.007</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Baseline 0.220±0.010</td>
<td>0.151±0.007*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days 0.227±0.012</td>
<td>0.155±0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 days 0.226±0.010</td>
<td>0.158±0.008</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD of 7 rats per group in g/cm$^2$. Bone mineral density (BMD) variations in male and female Control (Ctr) and suspended (S) rats during a 14-day experiment. Animals were measured weekly. $P < 0.05$ vs. (male); *age- and sex-matched Ctr.
paralleled that of dLS/BS during suspension. Neither N.Oc./B.Ar nor Oc.S./BS evolved differently during suspension.

**Differential Changes Between Males and Females in Responding to Suspension**

Bone loss was significantly more accentuated in suspended males (45% decrease in BV/TV) than in suspended females (15% decrease in BV/TV). This phenomenon was seen at the level of the overall femoral BMD and at different compartment sites in the proximal tibia, where BV/TV loss is fourfold higher in males than in females, mainly due to trabecular disappearance. In females, the 15% BV/TV loss was accompanied by a mild decrease in both Tb.Th and Tb.N. In both sexes the bone formation was similarly affected, whereas bone resorption was elevated in suspended males only. As for the tibia cortical area, males lost twice more than females.

**DISCUSSION**

We investigated whether sex influences the kinetics of disuse-induced bone loss in Wistar rats subjected to the hind-limb-unloaded model. We used 12-wk-old male and female rats, which matched with the age range of rats most usually used in both spaceflight and simulation experiments. We first compared bone phenotype over the experimental 2-wk duration period in male and female rats. The total BMD in the humerus and femur was similar in control male and female rats. How-
ever, tibial BMD was 16% higher in females at baseline, although the difference decreased over the 2 wk. Because the proximal tibia is one of the most frequently studied locations in the rat, μCT 3-D analysis was performed at this level. Major sex-related differences in the bone compartments were identified. The trabecular bone volume at the proximal metaphysis was twofold higher in females than in males at baseline, and it continued to increase, whereas in males it plateaued over the 2 wk. This higher BV/TV in females was accompanied by more numerous and more connected trabeculae, being less rod-like shaped than in males. Conversely, cortical area was 23% greater in males than in females, but in both sexes, cortical bone was still expanding during the experimental period. The greatest trabecular bone volume associated with smaller cortical area in females compared with males probably accounted for the relatively small difference in total BMD (18%) between sexes at this site. These results confirmed that there is no relation between body weight and BMD (9). They also led to the conclusion that, not only did the whole body and skeleton maturities differ between sexes, but each bone compartment had its own development. This might be linked to the occurrence of growth plate closure that differs between the sexes, rat strains, and bone sites (28). Bone growth continues much longer after sexual maturity in rats than in other animals (20). It was also suggested that estrogens were involved in the higher bone mass in females, which is related to reproductive needs. Pregnancy in the rat appears to exert little influence on bone, whereas lactation induces significant bone loss, mainly in the area of predominant trabecular bone (33), which is partially or completely reconstituted in the postlactational period (5). In females, we found greater mineral apposition rate and lower active resorption surfaces in the secondary spongiosa of the tibial proximal metaphysis compared with males; these differences might account for the cancellous tissue differences between the sexes. In Fischer 344 rats, females also had a greater trabecular bone volume and a higher bone formation rate than males in the proximal metaphysis of tibia (13). In 1- to 1.5-mo-old Sprague-Dawley rats, lower resorption rates along with greater bone mass were reported in females compared with males in the axial skeleton but not in cranial and appen-

Table 2. Bone cellular activities in the secondary spongiosa of the tibial proximal metaphysis after 14 days

<table>
<thead>
<tr>
<th>Group</th>
<th>MAR, μm/day</th>
<th>dLS/BS, %</th>
<th>BFR/BS, μm²/μm²/day</th>
<th>Oc.S/BS, %</th>
<th>N.Oc/B.Ar, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctr</td>
<td>2.60 ± 0.12</td>
<td>7.93 ± 2.54</td>
<td>0.21 ± 0.07</td>
<td>15.69 ± 2.48</td>
<td>115.65 ± 15.67</td>
</tr>
<tr>
<td>S</td>
<td>2.16 ± 0.12</td>
<td>3.99 ± 1.28</td>
<td>0.086 ± 0.03*</td>
<td>14.93 ± 3.34</td>
<td>162.56 ± 8.41*</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctr</td>
<td>3.44 ± 0.37δ</td>
<td>8.30 ± 1.42</td>
<td>0.29 ± 0.06</td>
<td>8.12 ± 1.70δ</td>
<td>93.93 ± 18.46</td>
</tr>
<tr>
<td>S</td>
<td>2.96 ± 0.54δ</td>
<td>4.30 ± 2.20a</td>
<td>0.13 ± 0.07*</td>
<td>10.14 ± 2.15δ</td>
<td>110.83 ± 13.71δ</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD of 7 rats per group. Bone formation (MAR, dLS/BS, BFR/BS, see text for nomenclature) and bone resorption (Oc.S/BS, N.Oc/B.Ar, see text for nomenclature) parameters in Ctr and S rats. P < 0.05 vs. δ; *, age- and sex-matched Ctr.
Sexual dimorphism may result from different growth rates between the sexes, where one sex develops faster than the other in the same time interval. In the Wistar-Han rats of both sexes, sexual maturity appeared to occur at the same age between 7 and 8 wk (Ref. 32 and Charles River Laboratories, personal communication), suggesting that the onset of reproduction is not associated with age-related differences in skeletal maturation.

The increasing degree of anisotropy observed in control males was even more accentuated in suspended animals, whereas the growth-related decreased degree of anisotropy occurring in females was not altered by unloading. This fact might be related to the decreased bone formation rate seen in male and female suspended rats.

In the study of Hefferan et al. (13), bone differences at the proximal metaphysis of the tibia between males and females appeared similar to those found in the present study. However, we have not found important changes in Tb.Th, as Hefferan et al. (13) did, and this was probably due to a lack of correlation between histomorphometric and μCT evaluation for this parameter (7). Hindlimb unweighting resulted in different responses mainly concerning cortical macroscopic parameters and lack of sex specificity in the responses of cancellous compartment. The disparity between their results and ours might be explained by the fact that, as reported for mice (2), there are strain-related differences in skeletal adaptation (inbred Fisher 344 vs. outbred Wistar-Han rats).

Globally, hindlimb unloading reversed in males the aging-related evolution for the following parameters: trabecular thickness, SMI, and cortical area, and it accentuated the aging-related changes for cancellous bone volume, trabecular number, connectivity density, and degree of anisotropy. In females, unloading reversed all the growth-related changes except for the degree of anisotropy. Both cortical and cancellous compartments were affected by suspension in males and females, although more extensively in males. We conclude that mechanical stimulus is a primary regulator over maturity stage even if estrogen-driven extracondensation of bone during puberty in the female skeleton might dampen its responsiveness to mechanical loading. In older female rats (6 mo), bone loss after hindlimb unloading was evidenced only at the cortical level (1), suggesting that trabecular bone sensitivity to unloading decreases with aging.

In conclusion, we have shown that longitudinal survey is required to allow comparison of time-dependent bone changes in control and experimental groups. The cortical tibia is still growing in both sexes at 3 mo. At these sites, males lose more bone than females. However, the cancellous compartment of the proximal tibia showed completely different profiles in males, already in a plateau phase, and in females, still in a growing process. Immobilization reversed the growth evolution in females and accentuated the aging evolution in males. Future studies have to address the question of the extent and mechanisms of unloading-related bone alterations according not only to sex but also to skeletal maturity.

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