Mechanical loading attenuates bone loss due to immobilization and calcium deficiency

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Inman, Cynthia L., Gordon L. Warren, Harry A. Hogan, and Susan A. Bloomfield. Mechanical loading attenuates bone loss due to immobilization and calcium deficiency. J. Appl. Physiol. 87(1): 189–195, 1999.—Our purpose was to determine the effects of a mechanical loading intervention on mass, geometry, and strength of rat cortical bone during a period of disuse concurrent with calcium deficiency (CD). Adult female rats were assigned to unilateral hindlimb immobilization, immobilized-loaded, or control (standard chow, 1.85% calcium) treatments. Both immobilized groups were fed a CD rat chow (0.01% calcium) to induce high bone density; adult rats; disuse; mechanical properties

bone loss; adult rats; disuse; mechanical properties

OSTEOPOROSIS is a multifactorial disease, with contributions from genetic factors, lack of physical activity, inadequate nutritional intake, and endocrine deficiencies. For example, the declines in vitamin D status, dietary calcium intake, and calcium absorption efficiency typically observed in the elderly may account, in part, for the increases in serum parathyroid hormone (PTH) observed in many older individuals (7). Chronically elevated serum PTH can contribute to increased rates of bone resorption and loss of bone mass at the femoral neck in the elderly (16). Many older individuals also decrease voluntary physical activity or may even be subjected to prolonged bed rest, further exacerbating bone loss (11).

The decrease in mechanical loading incurred with experimental limb immobilization results in localized bone loss that is similar in many respects to that observed with osteoporosis (12–14, 20). Unilateral hindlimb immobilization in the adult rat has several advantages as a model for postmenopausal or aging-related bone loss. Immobilization effectively stimulates resorption to exceed the rate of bone formation, resulting in a loss of both cancellous and cortical bone (12, 13). If calcium deficiency is superimposed on the effects of disuse, the rate of bone loss is accelerated because of the resultant increase in serum PTH (20, 23). To our knowledge, only one previous study has addressed the combined effects of calcium deficiency and immobilization in skeletally mature rats (20).

If this disuse-induced loss of bone mass could be slowed or prevented altogether, one would expect that significant decrements in bone strength and the increased risk of fracture could be minimized. Because weight-bearing activities can slow the loss of bone with aging (9, 17), mechanical loading during a period of disuse might also be effective in minimizing deleterious changes in bone mass and bone geometry affecting fracture risk. Several means of producing quantifiable mechanical loading have been developed for use in animal models (4, 10, 19). The purpose of this investigation was to test the hypothesis that in vivo mechanical loading in adult female rats can attenuate losses of cortical bone mass and strength during unilateral hindlimb immobilization concurrent with calcium deficiency.

METHODS

Experiment 1. Twenty-eight female Sprague-Dawley retired breeder rats, aged 5 mo (300 ± 3 g; Harlan, Indianapolis, IN) were randomly assigned to one of three groups: control, immobilized, and immobilized-loaded. The control rats (n = 9) were fed a standard rat chow (1.85% calcium, Harlan Teklad, Madison, WI) and were free to move about their cages without restriction. After a 1-wk acclimation period, four animals consuming normal rat chow were killed to serve as the baseline control. The right hindlimb of rats in the immobilized group (n = 9) was immobilized for 6 wk by taping, as previously described (13); this group was simultaneously fed a calcium-deficient diet (TD no. 93278; 0.00% calcium, Harlan Teklad). The immobilized-loaded group (n = 10) experienced external mechanical loading of the immobilized tibia three times per week, beginning 1 wk after the start of immobilization. In addition, they were fed the calcium-deficient diet. All rats were housed two per cage; the lighting schedule was 12 h on, 12 h off, and deionized water was provided. Rats were allowed to consume their assigned rat chow ad libitum. Rat chow for each cage was weighed daily; total chow consumed per day was halved to estimate food consumption per rat. After 6 wk, all rats were killed by a fatal
dose of pentobarbital sodium (150 mg/kg). Left and right tibiae were removed, cleaned of soft tissue, and stored in saline at −80°C. These samples were used for densitometry and for mechanical testing. The left and right tibias anterior and soleus muscles, along with the spleen, were removed, and wet weights were recorded.

Experiment 2. Thirty female Sprague-Dawley retired breeder rats, aged 5 mo old (306 ± 4.1 g; Harlan Sprague Dawley) were randomly assigned to baseline control (n = 7), aging cage-activity control (n = 8), immobilized (n = 8), and immobilized-loaded (n = 7) groups. Control rats ate standard chow, and both immobilized groups consumed the calcium-deficient chow as described above. Ten and three days before euthanasia, all rats received intraperitoneal injections of calcine in saline (8 mg/kg; Sigma Chemical) at pH 6.8 to label mineralizing bone surfaces. After 6 wk, rats were killed by a fatal dose of pentobarbital sodium. The right tibiae were removed, cleaned of soft tissue, and stored in 70% ethanol at 4°C. These samples were used for histomorphometric analyses. The study protocol and animal procedures for both experiments met the guidelines of the Texas A&M University Laboratory Animal Care Committee.

Immobilization method. Before immobilization of the right hindlimb, rats were anesthetized with methoxyflurane (Metofane; Pitman-Moore), and each animal’s lower torso and right hindlimb were shaved of all hair. A protective skin coating (ALLKare protective barrier wipe, ConvaTec) was applied to the exposed skin. The right hindlimb was immobilized against the abdomen with the hip joint in flexion and the knee and ankle joints in extension, using four to five layers of elastic tape (Johnson & Johnson Elastikon). Within 24 h, the rats were able to ambulate on three legs with no obvious discomfort. Throughout the 6-wk treatment period the animals were checked daily for evidence of discomfort, sores, or swelling. It was necessary to regularly reapply the tape bandaging one to two times per week to maintain immobilization. During retaping the animal was anesthetized, and the leg was massaged and stretched before reapplication of the tape.

Mechanical loading. Commencing 1 wk after the start of immobilization, immobilized-loaded rats had their right hindlimb untaped and the tibia was subjected to external loading three times per week. The in vivo loading was accomplished by using a four-point loading device previously described (1). Briefly, bending loads were applied by a lever system powered by a stepper motor; the applied load (in N) was determined by measuring strains induced on a calibrated load cell. Applied external loads of ~32 N (bending moments of ~96 N/mm) generated peak strains of 1,000–1,400 microstrain (µε) on the tibia’s lateral surface. These strain magnitudes are biologically relevant because significant bone modeling responses have been demonstrated in bone experiencing these strain magnitudes in rats (14, 19), turkeys (10), dogs (3), and sheep (5).

On loading days, each rat was anesthetized, the tape was removed, and the immobilized limb was stretched: the rat’s right hindlimb was then loaded for 36 cycles at 2 Hz with an external load of 32 N. A similar regimen has been shown to produce new bone formation on the medial periosteal surface of the tibial diaphysis after 3 wk of alternate-day loading (19). After each loading protocol was complete, the rat’s hindlimb was retaped, and the rat was returned to its cage. At no time during this procedure was the rat allowed to bear weight on the immobilized hindlimb.

Densitometry. The Norland XR 2600 dual-energy X-ray absorptiometer (Norland, Ft. Atkinson, WI) with Small Subject software (version 2.5, Norland) was used to estimate bone mineral density (BMD) and bone mineral content (BMC) in three regions of interest on both immobilized (right) and weight-bearing (left) tibiae. Thawed bones, cleaned of soft tissue, were placed on top of a Lucite phantom block, medial side down. The proximal tibia region included the full width of the most proximal 8 mm of the tibia. The midshaft tibia region extended from 4.0 to 13.5 mm proximal to the tibiofibular junction, including the full width of the tibial diaphysis, corresponding to that region experiencing bending strains during the in vivo four-point bending (Fig. 1). The distal tibia region included the full width of the most distal 6 mm of each bone. Scanning speed was 2 mm/s, with resolution set at 0.5 × 0.5 mm. Coefficients of variation were determined from five repeat scans on two tibiae over several days, with repositioning for each scan. The average coefficients of variation across all three regions were 2.8% for BMC and 2.2% for BMD.

Mechanical testing. Cortical bone strength at midshaft was determined by using a three-point bending-to-failure procedure on an Instron machine (model 1125). The area tested was at the midpoint of the region loaded during in vivo four-point bending. The tibia was positioned lateral side down on the custom-made supports (pins 4 mm in diameter) positioned 18 mm apart. A 50-lb. load cell was used with quasi-static loading (2.54 mm/min) applied to the medial surface of the tibia at midshaft. The small displacements of the servo-controlled Instron were monitored by a linear variable differential transformer interfaced with a personal computer unit. Load vs. displacement plots were recorded by using Gardener Systems software. Ultimate load was defined as the highest load (N) recorded just before the first decline in load as displacement increased. Stiffness was determined as the slope of the linear portion of the load vs. displacement curve, using TableCurve 2.0 (Jandel Scientific, San Rafael, CA).

Cross-sectional geometry was determined by embedding the distal half of each bone in black polyester resin. Transverse sections were cut by a low-speed diamond wafering saw (Buehler Isomet, Lake Bluff, IL). The bones consistently fractured beneath the upper loading point in the middiaphysis region, and each section was cut as near to the fracture point as possible. A Wild M420 macroscope was used to capture the image of the cross-sectional image by using Bioscan Optimas software. Jandel SigmaScan software (version 1.20.09) was used to determine cross-sectional moment.

Fig. 1. Loaded region of tibia with in vivo 4-point bending extends from 3.5 to 14.5 mm proximal to tibiofibular junction (TFJ). Lateral surface experiences compression, medial surface tension, in bending. Loading is applied on alternate days, 36 cycles at 2 Hz; strains on lateral surface as measured by uniaxial strain gauges were ~1,200 microstrain (µε) (Inman and Bloomfield, unpublished observations). Stippled area, scanned for midshaft bone mineral density (BMD) and bone mineral content (BMC; experiment 1); solid area, separate tibiae sectioned for histomorphometric analyses (experiment 2; 5–7 mm proximal to TFJ).
of inertia (CSMI) about the anterior-posterior axis. Determination of modulus of elasticity (E) was estimated by employing classic beam theory by using the equation $E = \frac{[(K\cdot L^3) - 1,000]}{48\cdot CSMI}$, where $K$ is stiffness and $L$ is bottom support span (18 mm). Ultimate stress ($\sigma$) of each sample was calculated by using the equation $\sigma = \frac{(UL\cdot L\cdot OD)}{(8\cdot CSMI)}$, where $UL$ is ultimate load, $L$ is bottom support span (18 mm), and $OD$ is outer diameter of bone at failure site.

Histomorphometry. The right tibiae from rats in experiment 2 were block stained in Villanueva stain (Polysciences) for 72 h and then progressively dehydrated in ethanol and acetone before embedding in methyl methacrylate. Cross sections of 120–150 µm were cut on a low-speed diamond wafer saw (Buehler Isomet) starting 5 mm proximal to the tibiofibular junction, which is centered in the region of maximal bending for those tibiae receiving mechanical loading. After sections were ground to a thickness of 80–100 µm and mounted on slides, image analysis for areas and labeled perimeters was performed by using BioQuant TrueColor Windows, version 2.0 (R&M Biometrics, Nashville, TN). Total tissue area (inside periosteal perimeter) and marrow area (Ma.Ar) were measured at an objective lens magnification of ×2; cortical bone area (Ct.Ar) was calculated as the difference between these two areas. Each cross section was divided into medial and lateral regions as previously described (19). By using epifluorescent illumination, total periosteal and endocortical perimeters and single-labeled perimeters were quantified for each region. No double-labeled perimeters were observed on cortical bone surfaces in all samples; therefore, we could not calculate bone formation rate or mineral apposition rate. Uptake of fluorochrome labels was confirmed by the presence of double labels in cancellous bone samples from the same animals. Samples from two control rats showed no evidence of calcine uptake and were excluded from the determination of single-labeled perimeters. Single-labeled perimeters are expressed as a percentage of the total bone perimeter. All histomorphometric nomenclature conforms to that recommended by Parfitt et al. (18).

Statistical analyses. Group differences were evaluated by using one-way ANOVA. When a significant main effect was detected, group means were further compared by using Student-Newman-Keuls or Duncan's multiple-range post hoc tests. If normality or equal variance assumptions were violated, a Kruskal-Wallis ANOVA on ranks was performed. Unpaired $t$-tests were performed on data from the baseline control rats and the aging control rats in both experiments to assess changes over 6 wk of aging. An alpha level of $P < 0.05$ was used to determine statistical significance. All statistical tests were done by using SigmaStat software (Jandel Scientific).

RESULTS

There were no significant differences between the two control groups in experiment 1 (baseline and aging control) for most variables. Therefore, results from the two control groups were pooled (n = 9 after pooling) for the remainder of the statistical analyses in both experiments for all but the histomorphometric variables. One immobilized-loaded rat in experiment 2 died prematurely because of an overdose of methoxyflurane during an external loading and retaping procedure. Immobilized and immobilized-loaded groups experienced similar weight loss during the experimental period. Total weight loss over 6 wk amounted to a mean of 54.5 ± 3.7 g in the immobilized group and 42.3 ± 6.6 g in the immobilized-loaded group (Fig. 2A). Food consumption in both immobilized groups was significantly less than that of the control group (Fig. 2B). Observations during daily monitoring suggested reduced voluntary physical activity of immobilized animals. Given the different body weights of immobilized groups vs. control rats, tissue weights are expressed relative to total body weight (Table 1). Normalized spleen weight did not vary among groups. Effective immobilization was confirmed by the 43 and 50% decrements in tibialis anterior and soleus muscle normalized weights, respectively, of the right hindlimb (Table 1). The loading regimen had no effect on muscle or spleen weights.
BMD and BMC of the immobilized rats’ tibial midshaft were 12 and 10% lower, respectively, than those of control rats (Table 2). The proximal tibia appeared most affected by immobilization, with 23–28% reductions in BMD or BMC vs. control. At the distal tibia BMC was unaffected, whereas BMD was reduced by 10%. Five weeks of mechanical loading prevented the decline in both BMD and BMC of the tibial midshaft seen with immobilization. The decreases in BMD and BMC were proportionately smaller than were the decreases in total body weight, as evidenced by 6–19% increases in BMD and BMC per gram of body weight in these groups. The loading regimen utilized did not alter BMD or BMC of the proximal or distal tibia.

Measures of mechanical strength and geometry of the tibial midshaft are illustrated in Fig. 3. Ultimate load of the tibia determined in three-point bending to failure was halved by the loading regimen. A similar pattern is observed for changes in stiffness. Loading effectively attenuated the 20% decline in stiffness observed with immobilization alone, reducing this deficit in stiffness to −8.5%, which was not significantly different from the value for control rats. Intergroup comparisons of CSMI nearly achieved significance (P = 0.056). CSMI in the immobilized and immobilized-loaded groups was 20 and 8% lower, respectively, than was mean CSMI in control animals. There were no significant differences among the three groups for modulus of elasticity nor for ultimate stress at the site of failure in these specimens.

Histomorphometric analyses (Table 3) of tibial middiaphysis from age-matched rats in experiment 2 allowed for tibial bone Ct.Ar to be determined in a consistent anatomic location (5–6 mm proximal to the tibiofibular junction), which was within 2 mm of the fracture site for those tibiae subjected to three-point bending to failure. Ct.Ar was 7% lower in immobilized tibiae than in aging control rat tibiae (P < 0.05), whereas loading of immobilized tibiae effectively maintained Ct.Ar. Ma.Ar at this site increased significantly in both immobilized groups, with a greater increase observed in the immobilized-loaded tibiae. Although increases in periosteal and endocortical surfaces exhibiting single fluorochrome label (%single-labeled surface) in immobilized-loaded tibiae ranged from 25 to 110% vs. unloaded immobilized tibiae, none of these increases was statistically significant.

Table 1. Body and tissue weights (normalized to body weight) of rats at euthanasia

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>R. tibialis ant.</th>
<th>L. tibialis ant.</th>
<th>R. soleus</th>
<th>L. soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>307 ± 5</td>
<td>1.99 ± 0.03</td>
<td>1.99 ± 0.04</td>
<td>0.42 ± 0.01</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Immob</td>
<td>8</td>
<td>248 ± 6*</td>
<td>1.13 ± 0.08*</td>
<td>1.95 ± 0.02</td>
<td>0.21 ± 0.01*</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>Immob-Load</td>
<td>10</td>
<td>253 ± 4*</td>
<td>0.96 ± 0.07*</td>
<td>1.91 ± 0.05</td>
<td>0.20 ± 0.01*</td>
<td>0.45 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; BW, body weight; Control, cage activity controls, killed at 0 and at 6 wk; Immob, right hindlimb immobilized for 6 wk; Immob-Load, immobilized tibia subjected to mechanical loading 3 times/wk; R, right; L, left; ant., anterior. *P < 0.05 vs. Control.

Table 2. Bone densitometry data on proximal, midshaft, and distal tibia of the immobilized hindlimb

<table>
<thead>
<tr>
<th>Group</th>
<th>BMC, mg</th>
<th>BMC, mg/g BW</th>
<th>BMD, mg/cm²</th>
<th>BMD, mg·cm² ·g BW⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal tibia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76 ± 3</td>
<td>0.25 ± 0.01</td>
<td>133 ± 4</td>
<td>1.0 ± 1.5</td>
</tr>
<tr>
<td>Immob</td>
<td>55 ± 2*</td>
<td>0.22 ± 0.01</td>
<td>102 ± 3*</td>
<td>−12.4 ± 10.0*</td>
</tr>
<tr>
<td>Immob-Load</td>
<td>58 ± 1*</td>
<td>0.23 ± 0.005</td>
<td>108 ± 2*</td>
<td>−11.5 ± 15.5*</td>
</tr>
<tr>
<td>Midshaft tibia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>48 ± 1</td>
<td>0.16 ± 0.01</td>
<td>95 ± 2</td>
<td>−0.4 ± 1.6</td>
</tr>
<tr>
<td>Immob</td>
<td>43 ± 1*</td>
<td>0.17 ± 0.004</td>
<td>84 ± 2*</td>
<td>−7.9 ± 11.1*</td>
</tr>
<tr>
<td>Immob-Load</td>
<td>48 ± 1†</td>
<td>0.19 ± 0.004†</td>
<td>94 ± 1†</td>
<td>−0.2 ± 1.2†</td>
</tr>
<tr>
<td>Distal tibia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31 ± 1</td>
<td>0.10 ± 0.003</td>
<td>105 ± 2</td>
<td>−0.2 ± 1.3</td>
</tr>
<tr>
<td>Immob</td>
<td>30 ± 1</td>
<td>0.12 ± 0.01*</td>
<td>94 ± 2*</td>
<td>−7.1 ± 13.3*</td>
</tr>
<tr>
<td>Immob-Load</td>
<td>29 ± 1</td>
<td>0.12 ± 0.002*</td>
<td>100 ± 2*</td>
<td>−4.7 ± 12.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMC, bone mineral content; BMD, bone mineral density. *P < 0.05 vs. Control. †P < 0.05 vs. Immob.
DISCUSSION

Acute calcium deficiency concurrent with immobilization effectively ensures a high turnover state in bone due to elevated serum PTH. The subsequent loss of bone with this combined disuse and calcium deficiency is dramatic; in immature rats (2 mo old), femoral ash weight declines by 46% after only 72 h (23). The age of the animal at the onset of immobilization and calcium deficiency appears to have a large impact on the magnitude and rapidity of the response. We observed smaller decreases of 28 and 10% in proximal tibial BMC and midshaft BMC, respectively, in our 5-mo-old rats subjected to immobilization and a low-calcium diet. The larger effect at the proximal tibia is likely due to the higher content of cancellous bone at this site, which experiences a more dramatic decrease in bone volume than does cortical bone with prolonged immobilization or spaceflight (15, 22). We also observed increased Ma.Ar, suggesting increased endocortical resorption, and a significant 6.5% decrease in Ct.Ar after 6 wk of this treatment.

The most significant findings of these studies are that mechanical loading imposed on immobilized cortical bone experiencing high turnover effectively maintained BMD and tibial cross-sectional area in that region experiencing bending forces during loading. Furthermore, the loss of mechanical strength normally observed with disuse was attenuated with this loading regimen. BMD remained significantly depressed at the proximal and distal tibial sites in immobilized hindlimbs undergoing in vivo four-point bending. Resorption at the endocortical surfaces persisted or increased in immobilized-loaded tibiae, as suggested by marrow cavity areas that were larger than in unloaded immobilized tibiae. This endocortical expansion was counterbalanced in the loaded bones by a suggestion of increased bone formation activity (implied from single-labeled surfaces) at the medial periosteal surface. In ambulatory rats, this same loading regimen produces a rapid increase in bone formation in the maximum bending area of the tibiae after only 2 wk of daily loading (6, 19).

In a comparison of the bending moments between in vivo four-point loading (estimated at 96 N/mm) and in vitro three-point bending before failure (estimated at 360 N/mm), it was determined that the strains produced during in vivo loading were within the elastic portion of the load-deformation curve. Hence our four-point bending regimen imposed loads within the rat tibia's usual functional loading ranges. In adult rats not subjected to immobilization, this loading procedure induces compressive strains on the lateral tibial surface of 1,000–1,400 με (Linnan and Bloomfield, unpublished observations), ~50% higher than strains produced during normal ambulation in the rat (8).

This loading intervention maintained bone cross-sectional area and possibly geometry (i.e., CSMI), thereby contributing to the maintenance or attenuated decline in bone mechanical properties during a period of immobilization. The decrease in ultimate load of midshaft tibial bone seen with immobilization was halved by the in vivo four-point bending regimen; tibial stiffness was effectively maintained. For small animals such as the rat, stiffness of appendicular bones may be the more important factor, because bone stiffness is critical in optimizing muscle function during locomotion and the peak loads typically imposed on the bone are small (2).

When ultimate load and stiffness (structural properties) are normalized to cross-sectional geometry, the resulting indexes (ultimate stress and modulus) yield information about material properties of the tissue independent of area or CSMI. There were no significant differences for modulus of elasticity or for ultimate stress with immobilization or with immobilization plus loading. If bone material properties are unchanged in the face of declining whole bone stiffness, the remaining possibility is that the quantity and/or geometry of the bone changed with disuse and calcium deficiency. The mean Ct.Ar declined 7% in immobilized tibiae. Shen et al. (20) observed a similar reduction (~9%) in Ct.Ar and a 44-fold increase in endocortical eroded surface in denervated hindlimbs of adult rats fed a calcium-deficient diet. Loading in the present study was effective in preventing the decline in Ct.Ar due to disuse and calcium deficiency. The larger Ma.Ar in the immobilized-loaded animals vs. those in immobilized rats also imply greater endocortical resorption, peristea expansion, and larger CSMI. Although changes in bone CSMI among groups did not achieve statistical significance, the attenuated declines in ultimate load and stiffness seen with loading suggest that the smaller decline in CSMI in loaded tibiae vs. that in unloaded immobilized tibiae was functionally significant.

A similar beneficial effect of an external loading intervention has been demonstrated in functionally isolated (and therefore immobilized) avian cortical bone, in which the usual loss of bone area was exacerbated by
dietary calcium deficiency. A daily loading regimen of 100 cycles generating 2,000 μe on bone surface effectively attenuated a 32% decrease in cross-sectional area observed in untreated isolated ulnae of egg-laying turkeys (10). In this model, no effect on bone formation was observed; the attenuated bone loss resulted from an inhibition of resorption, on the basis of radiographic evidence. A similar attenuation of resorptive activity during immobilization has been demonstrated in functionally isolated cortical bone in dogs exposed to pulsed electromagnetic fields for 1 h/day (21). Our study results suggest a modest stimulation of periosteal bone formation with external loading of the immobilized rat tibia as a potential mechanism for the maintenance of bone area. It is unclear why estimated resorption activity at the endocortical surface continued unabated with the external loading regimen we used.

We observed that the rats subjected to unilateral hindlimb immobilization were much less active than the control group. This lack of activity may account for their lower food consumption vs. that by the control rats. Similar body weight changes have been reported in tape-immobilized rats by Maeda et al. (14). Even though the immobilized animals undergoing external loading procedure three times weekly were exposed to methoxyflurane anesthesia for <10 min at a time, it is possible that decreased appetite was a residual effect of the frequent anesthesia. This seems unlikely, however, as immobilized rats not undergoing the mechanical loading procedure were subjected to anesthesia less often but lost body weight at an identical rate.

The important findings of this study are that mechanical loading as provided by four-point bending in vivo did attenuate the loss of cortical bone strength and effectively maintained bone density and cross-sectional area in immobilized bone experiencing high turnover. Our results expand on previous findings that the net balance between resorption and formation (and therefore bone mass) in cancellous bone is influenced by present levels of mechanical loading, whereas endocrine factors such as estrogen control the rate of bone turnover (24). In our study, bone sites distant from the loaded region still lost bone mineral during the immobilization period. Developing therapeutic loading regimens for use in humans experiencing multiple risk factors for osteoporosis, such as prolonged bed rest and insufficient calcium intake, should help minimize losses in bone strength and the attendant increase in fracture risk.

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