Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone

Russell T. Turner,1 Sutada Lotinun,1 Theresa E. Hefferan,2 and Emily Morey-Holton3

1Department of Nutrition and Exercise Science, Oregon State University, Corvallis, Oregon; 2Department of Orthopedics, Mayo Clinic College of Medicine, Rochester, Minnesota; and 3National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California

Submitted 27 December 2005; accepted in final form 19 April 2006

Turner, Russell T., Sutada Lotinun, Theresa E. Hefferan, and Emily Morey-Holton. Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone. J Appl Physiol 101: 881– 886, 2006. First published May 4, 2006; doi:10.1152/japplphysiol.01622.2005.—Intermittent treatment with parathyroid hormone (PTH) increases bone formation and preserves bone loss in hindlimb-unloaded (HLU) rats. However, the mechanisms of action of PTH are incompletely known. To explore possible interactions between weight bearing and PTH, we treated 6-mo-old weight-bearing and HLU rats with a human therapeutic dose (1 μg·kg−1·day−1) of human PTH(1–34) (hPTH). Cortical and cancellous bone formation was measured in tibia at the diaphysis proximal to the tibia-fibula synostosis and at the proximal metaphysis, respectively. Two weeks of hindlimb unloading resulted in a dramatic decrease in the rate of bone formation at both skeletal sites, which was prevented by PTH treatment at the cancellous site only. In contrast, PTH treatment increased cortical as well as cancellous bone formation in weight-bearing rats. Two-way ANOVA revealed that hPTH and HLU had independent and opposite effects on all histomorphometric indexes of bone formation [mineral apposition rate (MAR), double-labeled perimeter (dLPm), and bone formation rate (BFR)] at both skeletal sites. The bone anabolic effects of weight bearing and hPTH on dLPm and BFR at the cortical site were additive, as were the effects on MAR at the cancellous site. In contrast, weight bearing and hPTH resulted in synergistic increases in cortical bone MAR and cancellous bone dLPm and BFR. We conclude that weight bearing and PTH act cooperatively to increase bone formation by resulting in site-specific additive and synergistic increases in indexes of osteoblast number and activity, suggesting that weight-bearing exercise targeted to osteopenic skeletal sites may improve the efficacy of PTH therapy for osteoporosis.

Bone anabolic; aging; bone remodeling; exercise

INTERMITTENT, USUALLY ONCE daily, administration of parathyroid hormone (PTH) is capable of increasing bone mass in osteoporotic postmenopausal women (6, 18, 22). Similarly, PTH can reverse bone loss in moderately osteopenic ovariec-
tomized rats (24, 26). Estrogen is a potent inhibitor of bone turnover, but neither estrogen nor estrogen analogs have been reported to antagonize the bone anabolic response to intermittent PTH treatment in women or female rats (5, 16, 26). Although these results suggest that estrogen is neither essential for nor detrimental to the bone anabolic response to PTH, not all patients respond positively to PTH treatment (2, 11). This important observation indicates that genetic and environmental factors influence the bone anabolic response to PTH. Identifi-

Address for reprint requests and other correspondence: R. T. Turner, Dept. of Nutrition and Exercise Sciences, 107 Milam Hall, Oregon State Univ., Corvallis, OR 97333 (e-mail: russell.turner@oregonstate.edu).

http://www.jap.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
daily with recombinant hPTH(1–34) (1 μg·kg−1·day−1 sc) (a gift from Eli Lilly, Indianapolis, IN) or vehicle (0.1 ml 2% heat-inactivated rat serum in acidified saline) for the entire 14-day study.

To assess bone formation, animals were labeled by subcutaneous injection at the base of their tail with fluorochromes. All animals received a baseline label on day 0 of the experiment (20 mg/kg oxytetracycline; Sigma Chemical, St. Louis, MO). The baseline animals were killed 24 h later on day 1. The HLU groups were unloaded on day 1. The second fluorochrome label, 10 mg/kg calcein (Sigma Chemical), was administered 10 days before death, and the third fluorochrome, 10 mg/kg calcein, was given 2 days before death. At the end of 14 days of unloading, the animals were weighed, anesthetized by CO2, and decapitated. The seminal vesicle was excised and weighed. The right tibia was excised and fixed in 70% ethanol. Tibia length was measured using a precision caliper, and the bone was weighed. The right tibia was excised and fixed in 70% ethanol. Tibia length was measured using a precision caliper, and the bone was processed for histomorphometry.

Bone histomorphometry. The histomorphometric measurements were performed using the OsteoMeasure Analysis System (OsteoMetrics, Atlanta GA), as has been described (31).

Cortical bone histomorphometry. Ground sections were cut proximal to the tibia-fibula synostosis and prepared as described (31): cross-sectional area, medullary area, cortical bone area, periosteal double-labeled perimeter (dLpM), periosteal mineral apposition rate (MAR), and periosteal BFR were measured.

Cancellous bone histomorphometry. The tibial metaphysis was dehydrated and embedded without demineralization to preserve the fluorochrome labels and sectioned at a thickness of 5 μm (31). The oxytetracycline label was located within the calcified growth-plate cartilage, indicating that there had been minimal longitudinal bone growth. An area of 2.8 mm2, 1 mm from the growth plate in proximal tibial metaphysis, was analyzed to obtain the following static bone measurements and calculated values were obtained as described (31): bone area normalized to tissue area (BA/TA), trabecular number (TbN), trabecular thickness (TbTh), and trabecular separation (ThSp). The following dynamic bone measurements and calculated values were obtained as described (31): dLpM, MAR, and BFR normalized to bone perimeter (BpM).

Statistical analysis. Comparisons between weight-bearing controls and the baseline and experimental groups were made by ANOVA using StatView statistical software (Abacus Concepts, Berkeley, CA). Post hoc comparisons were made by using the Fisher’s protected least significant test, with a statistical significance defined as P < 0.05. In addition, the respective effects of PTH treatment and HLU were determined by two-way ANOVA using Super ANOVA software (Abacus Concepts). Values are means ± SE.

RESULTS

Body weight, seminal vesicle weight, and tibia length are shown in Table 1. HLU resulted in a 21.5% decrease in body weight compared with the baseline value. hPTH treatment had no independent effect on body weight and did not influence the magnitude of weight loss resulting from HLU. Similarly, HLU resulted in a 25.4% decrease in seminal vesicle weight, whereas PTH had no independent effect and did not influence seminal vesicle weight loss in unloaded rats. Neither HLU nor treatment with hPTH had an effect on tibia length.

Static cortical bone measurements are shown in Table 2. Neither HLU nor treatment with hPTH had an effect on tibia cross-sectional area, medullary area, or cortical bone area, and there were no interactions between the two treatments.

Micrographs of representative diaphyseal sections visualized by UV microscopy are shown in Fig. 1. Dynamic cortical bone measurements are shown in Fig. 2. MAR is shown in Fig. 2A. HLU decreased, whereas hPTH increased MAR. There was an interaction between HLU and hPTH such that HLU prevented the hPTH-induced increase in MAR. dLpM is shown in Fig. 2B. HLU decreased, whereas hPTH treatment increased dLpM. The effects of the two treatments were additive; there was no interaction between HLU and hPTH. The periosteal BFR is shown in Fig. 2C. HLU decreased bone formation, whereas hPTH treatment increased bone formation. No interaction between hPTH and HLU was detected by two-way ANOVA, but one-way ANOVA revealed that hPTH was ineffective in restoring BFR in HLU rats to the level of weight-bearing animals.

Static cancellous bone measurements are shown in Table 3. HLU had no independent effect on the bone measurements, whereas hPTH treatment increased BA/TA, TbTh, and TbN, and decreased TbSp. There was an interaction between the two

Table 1. Effects of HLU and hPTH on cortical bone growth in 6-mo-old male rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>Control</th>
<th>hPTH</th>
<th>HLU</th>
<th>hPTH + HLU</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necropsy weight, g</td>
<td>403±8</td>
<td>380±9</td>
<td>370±16</td>
<td>312±6*</td>
<td>306±5*</td>
<td>0.0001 NS NS</td>
</tr>
<tr>
<td>Seminal vesicle weight, g</td>
<td>0.71±0.07</td>
<td>0.85±0.07</td>
<td>0.80±0.06</td>
<td>0.53±0.04*</td>
<td>0.57±0.04*</td>
<td>0.0001 NS NS</td>
</tr>
<tr>
<td>Tibia length, cm</td>
<td>4.4±0.1</td>
<td>4.2±0.1</td>
<td>4.3±0.1</td>
<td>4.4±0.1</td>
<td>4.3±0.1</td>
<td>NS NS NS</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7–10). hPTH, human parathyroid hormone; HLU, hindlimb unloaded; NS, not significant. *Significant compared with control (P < 0.05).

Table 2. Effects of HLU and hPTH on cortical bone architecture in 6-mo-old male rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>Control</th>
<th>hPTH</th>
<th>HLU</th>
<th>hPTH + HLU</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA, mm²</td>
<td>3.54 ± 0.15</td>
<td>3.48 ± 0.10</td>
<td>3.35 ± 0.11</td>
<td>3.45 ± 0.16</td>
<td>3.42 ± 0.10</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>CA, mm²</td>
<td>2.56 ± 0.14</td>
<td>2.51 ± 0.08</td>
<td>2.37 ± 0.13</td>
<td>2.54 ± 0.14</td>
<td>2.43 ± 0.09</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>MA, mm²</td>
<td>0.91 ± 0.13</td>
<td>0.97 ± 0.07</td>
<td>0.99 ± 0.06</td>
<td>0.91 ± 0.07</td>
<td>0.99 ± 0.04</td>
<td>NS NS NS</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7–10). CSA, cross-sectional area; CA, cortical area; MA, medullary area. *No significant differences compared with control were observed.
treatments, whereby unloading antagonized the hPTH-mediated increase in BA/TA and decrease in TbSp. Compared with baseline, hPTH increased BA/TA and TbTh in weight-bearing rats but had no significant effects on these parameters in HLU animals.

Micrographs of representative proximal tibial metaphyseal sections illuminated with UV light are shown in Fig. 3. Dynamic cancellous bone measurements are shown in Fig. 4. HLU decreased and hPTH treatment increased MAR (Fig. 4A), dLPm/BPm (Fig. 4B), and BFR/BPm (Fig. 4C). There was no interaction between PTH and unloading on MAR, but HLU reduced the PTH-induced increases in BFR/BPm and dLPm/BPm. One-way ANOVA revealed that hPTH treatment restored BFR/BPm, dLPm/BPm, and MAR in unloaded rats to values that did not differ from the weight-bearing controls.

**DISCUSSION**

The present study demonstrates that hPTH, at dose rates (1 μg·kg⁻¹·day⁻¹) much lower than routinely used (i.e., 80 μg·kg⁻¹·day⁻¹) in rodent models and similar to the therapeutic dose in patients, results in large increases in bone formation in healthy, normal weight-bearing adult male rats. However, disuse reduced the bone anabolic effects of low-dose hPTH treatment in cancellous bone and largely prevented them in cortical bone. These data suggest that, in rats, there are dose- and bone compartment-dependent synergistic and additive interactions between hPTH treatment and mechanical loading on bone formation.

Most rat studies using the HLU model have been performed using immature animals. Osteopenia quickly develops in growing rats but is due to a net decrease in bone formation (19). This relative osteopenia, which is due to the failure to accumulate as much bone as normal, differs from HLU adult rats, in which bone loss occurs as a result of a net increase in bone resorption (9). In the present study, no decrease in BA/TA was observed following HLU. This result is not unexpected, because of the short duration of the study. Longer duration studies (1 mo long) have confirmed a decrease in BA/TA occurs in 6-mo-old male HLU rats (R. T. Turner, unpublished observations). The BFR was reduced in tibia of unloaded rats
within 2 wk of HLU, indicating that decreased bone formation precedes and contributes to the bone loss. The reduction in bone formation was due to a combination of decreased MAR and decreased dLPm (9), and continued unloading would be expected to result in impaired bone mechanical properties.

A therapeutic dose of hPTH was qualitatively similar to the standard high dose in that treatment increased cancellous and cortical bone formation in weight-bearing animals. Similarly, the increases in bone formation in hPTH-treated rats resulted from increases in indexes of osteoblast activity (MAR) and osteoblast number (dLPm). High-dose PTH was not investigated in this study. Based on the literature and our published and unpublished data, treatment with therapeutic and high-dose hPTH increases MAR to a similar magnitude, whereas the high-dose treatment results in a larger increase in dLPm (24, 31). Dose-response studies will be necessary to confirm that osteoblast number and activity are differentially sensitive to the dose of PTH. If this apparent difference in the dose-response relationship also occurs in humans, then studies performed in animals using high-dose hPTH exaggerate the contribution of increased osteoblast number to the bone anabolic response to PTH therapy.

There is substantial evidence in rodents that mechanical loading of the skeleton potentiates the bone anabolic response to PTH (8, 10, 15, 21, 29). However, an absolute requirement for loading to facilitate the stimulatory effects of PTH on bone formation has not been demonstrated, and very few studies have been designed to distinguish between additive and synergistic interactions between loading and the hormone. In a study designed to address this issue, normal weight-bearing and hPTH treatment were shown to have additive effects on bone formation in 6-mo-old ovariectomized rats (31). However, the high dose rate of PTH (80 μg·kg⁻¹·day⁻¹) used in this HLU study, although typical for studies performed in ovariectomized rats with established bone loss (24), greatly increased bone formation compared with normal. As a consequence, TbTh was increased compared with normal weight-bearing animals. Thus it was not clear whether a similar relationship would occur at a therapeutic dose of PTH intended to maintain normal levels of bone formation during disuse. The present study clarifies this issue and demonstrates significant interactions between loading and PTH on cortical as well as cancellous bone formation.

### Table 3. Effects of HLU and PTH on cancellous bone architecture in 6-mo-old male rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>Control</th>
<th>hPTH</th>
<th>HLU</th>
<th>HLU + PTH</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA/TA, %</td>
<td>24.9±1.0</td>
<td>21.5±2.0</td>
<td>29.7±1.3†</td>
<td>23.2±0.9</td>
<td>24.4±1.5</td>
<td>NS 0.005 0.03</td>
</tr>
<tr>
<td>TbTh, μm</td>
<td>77.0±2.0</td>
<td>74.2±3.7</td>
<td>85.3±3.1†</td>
<td>74.9±1.5</td>
<td>76.6±2.3</td>
<td>NS 0.03 NS</td>
</tr>
<tr>
<td>TbN, mm⁻¹</td>
<td>3.25±0.12</td>
<td>2.88±0.21</td>
<td>3.49±0.16</td>
<td>3.01±0.09</td>
<td>3.19±0.18</td>
<td>NS 0.05 NS</td>
</tr>
<tr>
<td>TbSp, μm</td>
<td>234±11</td>
<td>287±25*</td>
<td>204±13†</td>
<td>250±9</td>
<td>247±20</td>
<td>NS 0.04 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7–10). PTH, parathyroid hormone; BA, bone area; TA, tissue area; TbTh, trabecular thickness; TbN, trabecular number; TbSp, trabecular separation. *P < 0.05 compared with baseline; †P < 0.05 compared with control.

---

Fig. 3. Representative cancellous bone sections viewed under UV illumination. A: weight-bearing control. B: weight-bearing PTH treated. C: HLU. D: HLU PTH treated. The original magnification was ×200. Note the extensive fluorochrome labeling (arrows) in the weight-bearing control and PTH-treated animals.
Treatment with a therapeutic dose of hPTH prevented the hindlimb unloading-induced reduction in cancellous bone formation at the proximal tibia metaphysis. PTH was effective in maintaining dLpm and MAR at levels comparable to weight-bearing rats. Two-way ANOVA revealed that weight bearing and hPTH interacted to result in a synergistic increase in bone formation. The positive interaction between weight-bearing and hPTH treatment was even more dramatic in the diaphysis. Whereas hPTH treatment significantly increased bone formation at the periosteum in weight-bearing animals, the bone anabolic response to the hormone was largely absent in the HLU rats.

The site-specific additive and synergistic responses of the skeleton to weight-bearing and hPTH treatment may be a consequence of an overlap in the signal transduction pathways for mechanical signaling and the hormone. For example, IGF-I signaling is thought to be essential for the bone anabolic response to PTH, and PTH treatment increases IGF-I gene expression in bone (1, 23). Similarly, IGF-I gene expression in bone is associated with the stimulatory effects of weight bearing on bone formation (7, 12, 25). It is not clear how this hormonal and cytokine interaction would account for the pronounced site-specific differences in sensitivity to PTH demonstrated by the diaphyseal periosteum and metaphyseal cancellous bone sites in unloaded rats. One is tempted to speculate that the varied response may be related to the large differences in strain energies experienced at the two sites (32). Thus PTH may have a more dramatic effect at skeletal sites under high-strain energies because endogenous expression of key skeletal growth factors is favorable for maintenance of the osteoblast phenotype.

The large increase in osteoblast number on cancellous bone surfaces in PTH-treated rats is due to activation (phenotypic modulation) of postproliferative osteoblast lineage cells (e.g., lining cells and committed preosteoblasts) (4). Similarly, the initial increase in osteoblast number following ex vivo loading of a rat tibia does not involve cell proliferation. However, mechanical loading also results in a delayed response involving proliferation and differentiation of preosteoblasts (30). These observations support the possibility that there is overlap in the target cell populations and biochemical pathways mediating the skeletal response to PTH and normal weight bearing.

The dramatic decrease in bone formation in HLU rats is likely due to reduced dynamic loading of the bone. The precise mechanism by which one or more mechanical signals are transduced to biochemical signals that regulate bone metabolism is incompletely understood, despite intensive investigation. In addition to a reduction in mechanical stimulation of bone cells, HLU results in hormonal and metabolic changes that may influence bone metabolism. Serum testosterone, a known regulator of bone metabolism, has been reported to be decreased in male HLU rats (33). Similarly, uterine weight was decreased in pair-fed and HLU ovary-intact rats (9). Serum estradiol has not been reported in HLU male rats, but we have shown that orchiectomy has much larger effects on the male skeleton than the antiestrogen ICI 182,780 (27). This finding suggests that a reduction in androgen levels is more likely to influence bone metabolism in males than is a decrease in estrogens. We did not measure testosterone levels in this study, but in other studies (R. T. Turner, unpublished observations) we have shown them to be decreased in pair-fed and HLU rats.

In the present study, we measured seminal vesicle weight, a sensitive biomarker of androgen status, and found it to be decreased in HLU rats compared with baseline. However, seminal vesicle weight was decreased to a similar extent in pair-fed weight-bearing controls, suggesting that the observed decrease in sex steroid levels is sufficient to account for the skeletal changes in HLU rats.

The reduction in food intake contributed to the weight loss in HLU rats. Reduced food consumption may play a role in the adverse skeletal changes associated with HLU, because weight loss due to caloric restriction has been reported to result in bone loss (28). However, the control rats were pair-fed to the HLU animals, suggesting that reduced caloric intake was not a major factor in the observed decrease in bone formation in HLU rats. On the other hand, the greater weight loss in the HLU groups compared with the pair-fed control could indicate that unloading results in an increase in energy expenditure. The effect of HLU on energy expenditure and the role that altered metabolism may play in the skeletal response to disuse is the subject of ongoing studies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Glenda Evans and editorial assistance of Peggy Backup.

GRANTS

These studies were supported by grants from National Aeronautics and Space Administration (NAG9–1458) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR-48833).
REFERENCES


J Appl Physiol • VOL 101 • SEPTEMBER 2006 • www.jap.org