Parathyroid Hormone (1–34) Increased Total Body Bone Mass in Aged Female Rats

H. A. SIMMONS, C. M. PIRIE, D. D. THOMPSON and H. Z. KE
Osteoporosis and Frailty Research, Department of Cardiovascular and Metabolic Diseases, Pfizer Central Research, Groton, Connecticut
Accepted for publication March 31, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
Daily subcutaneous administration of bovine parathyroid hormone (PTH)(1–34) stimulates bone formation and increases bone mass in rat tibiae, femora and lumbar spine. However, the effects of PTH on the whole body bone mineral content and density determined by dual energy x-ray absorptiometry (DEXA) have not been previously reported in rats. Eighteen-month-old intact female rats were subcutaneously injected daily with 0, 40, 80 or 160 µg/kg/day of bovine PTH (1–34) for either 15 or 60 days. Whole body DEXA was performed at 1 day before autopsy, and bone area, bone mineral content (BMC) and bone mineral density (BMD) of the total body were determined. Total femoral, tibial and lumbar spine BMD was also determined ex vivo. Cancellous bone histomorphometry was performed on sections of double-labeled proximal tibial metaphyses. Whole body bone mineral content and density were significantly increased by 60 days, but not by 15 days, of PTH treatment at all dose groups compared with vehicle controls. Lumbar vertebral and total femoral BMD was significantly increased at all doses of PTH by 15 days of administration and further increased by 60 days. All doses of PTH increased trabecular bone area in proximal tibial metaphyses by 15 days and further increased by 60 days. In proximal tibial cancellous bone, dose-dependent increases in percent labeled perimeter, mineral apposition rate and bone formation rate-bone volume referent were found between 40 and 160 µg/kg of PTH treatment by 15 days, and no further increases were found by 60 days. Our results showed that in aged female rats, bovine PTH(1–34) increased bone formation and total body bone mass.

It has been reported that postmenopausal bone loss is not limited to spine and hip but occurs throughout the skeleton (Revilla et al., 1997). No anabolic agents are currently approved for the restoration of bone mass to the patients with established osteoporosis (Riggs et al., 1992). PTH when given by daily injection has been reported to increase vertebral mass in osteoporotic patients (Reeve et al., 1990), but its effects on cortical bone sites are unclear (Dempster et al., 1993).

The review of Dempster et al. (1993) cites numerous studies reporting on the anabolic activity of PTH. These studies conclude that PTH significantly increased trabecular and cortical bone mass when given intermittently in rats, under various treatment conditions (Liu et al., 1990; Kimmel et al., 1993; Mosekilde et al., 1994; Wronska et al., 1994; Jerome 1994; Li et al., 1995). While these studies have added greatly to our understanding of the anabolic action of PTH on bone in vivo, much of the literature with parathyroid hormone uses younger rats between 3 and 6 month old, focusing on specific bone sites. The few studies that have used older rats have focused on different aspects of the anabolic process (Dobni et al., 1995). Whole body calcium has been shown to increase after PTH treatment in rats as measured by ash weight and neutron activation (Hefiti, et al., 1982). However, the effects of PTH on total body bone mineral content and density have not been previously documented in aged rats using DEXA in combination with histomorphometric methods. Most studies have used only isolated bones ex vivo for determination of DEXA. We investigated the effect of bovine PTH(1–34) on total body bone and individual bone mass, as well as the static and dynamic histomorphometry of cancellous bone. Since a common dose of PTH(1–34) used in the previous animal studies was 80 µg/kg, we chose to bracket this with 40 and 160 µg/kg/day (Liu et al., 1990; Wronska et al., 1994). Two timepoints, 15 and 60 days, were used to observe the early and longer term response to this agent, as much of the literature found uses a single timepoint generally less than 6 weeks.

Materials and Methods
Sixty-four female retired breeder Sprague-Dawley rats (Charles River, Wilmington, MA) at 18 months of age were weighed and randomized into 8 groups for the daily subcutaneous injection of bovine PTH(1–34) at 0, 40, 80 or 160 µg/kg/day (Bachem, Torrance, CA) for either 15 or 60 days. The vehicle used was .001 N HCl + 1 mg/ml bovine serum albumin. All animals were individually housed and received for publication November 25, 1997.

ABBREVIATIONS: PTH, parathyroid hormone; DEXA, dual energy x-ray absorptiometry; TBV, trabecular bone volume; BFR, bone formation rate; MAR, mineral apposition rate; BMD, bone mineral density; BMC, bone mineral content.
and maintained on a 12-hr on/12-hr off light/dark cycle and were given food and water ad libitum (commercial diet-Agway ProLab 3000, Agway Country Foods, Syracuse, NY) (calcium 0.97%, phosphorus 0.85%, vitamin D₃ 1.05 IU/g). Dosing solutions were made fresh daily, and animals were dosed with 1 ml/kg of body weight. Animals were weighed weekly, and the injection volume adjusted accordingly. The experiment was conducted according to Pfizer Animal Care-approved protocols, and animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

All animals were given a subcutaneous injection of the fluorochrome calcein at 10 mg/kg (Sigma Chemical, St. Louis, MO) at 10 and 2 days before death. On the day before death, the animals were scanned for whole body bone area, mineral content and bone density measurements by DEXA, (Hologic QDR 1000/w, Hologic, Waltham, MA) equipped with a rat whole body scan software (Hagiwara et al., 1993). The scan field size was 15 × 8 cm, resolution was 0.0254 × 0.0127 cm and scan speed was 7.25 mm/sec. At both the 15- and 60-day timepoint, the animals were anesthetized, weighed and killed by cervical dislocation. The hindlimbs and lumbar vertebrae were removed at death and placed into 70% ethanol. The right femur, was used to compare the differences between groups (Neter et al., 1982). Linear regression analysis was used to determine dose-dependent response.

Results are expressed as mean ± S.E.M., and statistics are calculated vs. appropriate vehicle.

<table>
<thead>
<tr>
<th>N</th>
<th>Bone area</th>
<th>BMC</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>85.92 ± 0.89</td>
<td>12.99 ± 0.16</td>
</tr>
<tr>
<td>PTH, 40 µg/kg</td>
<td>7</td>
<td>85.28 ± 2.13</td>
<td>13.25 ± 0.48</td>
</tr>
<tr>
<td>PTH, 80 µg/kg</td>
<td>6</td>
<td>89.80 ± 2.92</td>
<td>14.20 ± 0.63</td>
</tr>
<tr>
<td>PTH, 160 µg/kg</td>
<td>7</td>
<td>89.51 ± 2.22</td>
<td>13.84 ± 0.26</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>90.59 ± 1.23</td>
<td>13.77 ± 0.23</td>
</tr>
<tr>
<td>PTH, 40 µg/kg</td>
<td>7</td>
<td>91.49 ± 2.79</td>
<td>14.82 ± 0.39</td>
</tr>
<tr>
<td>PTH, 80 µg/kg</td>
<td>7</td>
<td>93.67 ± 2.54</td>
<td>15.35 ± 0.46</td>
</tr>
<tr>
<td>PTH, 160 µg/kg</td>
<td>7</td>
<td>91.19 ± 2.62</td>
<td>15.55 ± 0.54</td>
</tr>
</tbody>
</table>

*P < .05.

Effects on proximal tibial BMC and BMD. In proximal tibial metaphyses, a significant increase in BMC was only found in rats treated with PTH at 80 µg/kg (+6.4%) by 15 days compared with controls. At 60 days, a significant increase in proximal tibial BMC of 13.2%, 15.5% and 19.1% was found in 40, 80 and 160 µg/kg of PTH-treated rats, respectively, compared with vehicle-treated controls at day 60. Linear regression analyses showed that there was no dose-dependent response in total femoral BMC and BMD in rats treated with PTH between doses of 40, 80 and 160 µg/kg/day (data not shown).

Effects on total femoral BMD. There was no significant difference in total femoral bone area among all groups at both days 15 and 60. The change in total femoral BMC was identical to that observed for the total femoral BMD; therefore, we present only the differences in total femoral BMC among the groups (table 2). Compared with vehicle-treated controls, there was a significant increase in total femoral BMC by 8.5%, 9.6% and 8.1% in rats treated with PTH for 15 days at 40, 80 and 160 µg/kg/day, respectively. At day 60, total femoral BMC was further increased by PTH treatment. Total femoral BMC significantly increased by 18.2%, 21.4% and 28.1% in rats treated with PTH at 40, 80 and 160 µg/kg/day, respectively, compared with vehicle-treated controls at day 60. Linear regression analyses showed that there was no dose-dependent response in total femoral BMC between the three doses of PTH administered at day 15. However, a significant dose-dependent increase in total femoral BMC was found by 60 days (r = .582, P < .001).

Effects on lumbar vertebrae BMD. At day 15, total lumbar vertebral BMD increased significantly by 7%, 10% and 10% in rats treated with PTH at 40, 80 and 160 µg/kg/day, respectively. By day 60, the vertebral BMD increased to 16%, 13% and 21% at 40, 80 and 160 µg/kg. Similarly, no significant difference was found among the three PTH doses (table 2).

Effect on proximal tibial histomorphometry. Histomorphometric data showed that parathyroid hormone at all doses increased the amount of trabecular bone. Trabecular bone volume was increased significantly for each of the doses at 15 (ranging from +56% to 79%) and 60 (+87% to 90%) days over vehicle, as was trabecular thickness (fig. 1). Trabecular number was increased at the 80 and 160 µg/kg doses at each timepoint (table 3). Compared with controls, all three doses of PTH significantly increased percent labeled perimeter.
bone resorption in these rats. Controls, indicating that PTH administered daily decreased significantly at both 15 and 60 days in rats treated with either 80 or 160 \( \mu g/kg \) of PTH compared with the same dose groups at day 15, percent labeled perimeter and BFR/BV at both 80 and 160 \( \mu g/kg \) of PTH treatment by 15 days. No further increase in these bone parameters was still significantly increased compared with the vehicle controls at 60 days. Osteoclast number/perimeter was significantly decreased at both 15 and 60 days in rats treated with either 80 or 160 \( \mu g/kg \) of PTH compared with vehicle controls, indicating that PTH administered daily decreased bone resorption in these rats.

**Discussion**

For the first time, we have shown that bovine PTH(1–34) is able to increase total body bone mineral content and density determined by DEXA in aged rats. In this study, PTH increased percent labeled surface and mineral apposition rate and therefore increased the bone formation rate at all doses administered. This increase led to an increase in trabecular bone volume and bone mass throughout the skeleton. Bone mineral content and density significantly increased in total body, total femora, lumbar vertebrae and proximal tibiae.

Parathyroid hormone increased bone mineral content and density, as measured by DEXA, in \textit{ex vivo} measurements of whole femur, proximal tibia and lumbar spine at both 15 and 60 days of treatment. However, bone area in these bone sites did not differ in PTH treated rats compared with controls, indicating that increased bone formation by PTH treatment in these aged female rats occurred mainly on the endocortical and trabecular surfaces, not on the periosteal surfaces. Thus, the outside diameter of the bone remained unchanged after PTH treatment. These results were further confirmed by whole body DEXA data where bone area did not change with PTH treatment at either 15 or 60 days.

Total body BMC and BMD significantly increased in rats treated with all doses of PTH as compared to controls at 60 days but not at 15 days. Therefore, there was a different response to 15 days of PTH treatment between total skeletal mass and bone mass in femora, tibia and lumbar vertebrae. Total body bone mass determined by \textit{in vivo} DEXA scans showed no significant effect of PTH, while total femoral, total lumbar vertebral and proximal tibial BMC and BMD, determined by \textit{ex vivo} DEXA scans, showed a significant increase by PTH treatment at day 15. This discrepancy may be due to the fact that the \textit{ex vivo} scan software for excised bones had higher resolution and sensitivity than that of rat whole body scan software. Thus, in \textit{ex vivo} analysis, the individual bone would appear to be more sensitive than that of the whole body scan. Another reason for this discrepancy may be that the other skeletal sites may not respond to PTH treatment as rapidly as the tibia, femur and spine, since these long bones and probably lumbar vertebrae may bear more mechanical forces than other skeletal sites (Frost, 1990a, 1990b). Nevertheless, significant increases in BMC and BMD of total body, total femora, proximal tibial and lumbar vertebrae were observed after 60 days of PTH treatment.

Proximal tibial cancellous histomorphometric analysis showed that PTH rapidly increased the percent labeled perimeter, mineral apposition rate and trabecular bone volume by 15 days at all doses administered. Similar findings were reported by Ma et al. (1995) in which they reported that 15 days of a higher dose (200 \( \mu g/kg/d \)) of human PTH(1–38)

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Whole femur</th>
<th>Lumbar spine</th>
<th>Proximal tibial metaphysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>0.274 ± 0.003</td>
<td>0.189 ± 0.003</td>
</tr>
<tr>
<td>PTH, 40 ( \mu g/kg )</td>
<td>7</td>
<td>0.297 ± 0.008a</td>
<td>0.203 ± 0.004a</td>
</tr>
<tr>
<td>PTH, 80 ( \mu g/kg )</td>
<td>6</td>
<td>0.300 ± 0.006a</td>
<td>0.208 ± 0.006a</td>
</tr>
<tr>
<td>PTH, 160 ( \mu g/kg )</td>
<td>7</td>
<td>0.296 ± 0.004a</td>
<td>0.208 ± 0.003a</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>0.282 ± 0.005</td>
<td>0.186 ± 0.003</td>
</tr>
<tr>
<td>PTH, 40 ( \mu g/kg )</td>
<td>7</td>
<td>0.333 ± 0.003a</td>
<td>0.215 ± 0.008a</td>
</tr>
<tr>
<td>PTH, 80 ( \mu g/kg )</td>
<td>7</td>
<td>0.342 ± 0.007a</td>
<td>0.210 ± 0.006a</td>
</tr>
<tr>
<td>PTH, 160 ( \mu g/kg )</td>
<td>7</td>
<td>0.382 ± 0.003a</td>
<td>0.225 ± 0.006a</td>
</tr>
</tbody>
</table>

* P < .05.

\( a \) P < .05.

Fig. 1. Effect of PTH on trabecular bone volume (A) and trabecular thickness (B) in aged female rats as measured on Bioquant computer-aided image analysis system. Results are expressed as mean ± S.E.M., and statistics are calculated vs. appropriate vehicle (+P < .05).

\((+210\% \text{ to } 456\%\), mineral apposition rate (+141\% \text{ to } 206\%)\) and bone formation rate (+359\% \text{ to } 962\%) by 15 days of administration (table 3). Linear regression analysis showed that there was a dose-dependent increase in percent labeled perimeter \( (r = .745, P < .001) \) and bone formation rate \( (BFR/BV) (r = .699, P < .001) \) between 40 and 160 \( \mu g/kg \) of PTH treatment by 15 days. No further increase in these bone formation indices was found by 60 days of treatment. Compared with the same dose groups at day 15, percent labeled perimeter and BFR/BV at both 80 and 160 \( \mu g/kg \) decreased significantly at 60 days of treatment, although these parameters were still significantly increased compared with the vehicle controls at 60 days. Osteoclast number/perimeter was significantly decreased at both 15 and 60 days in rats treated with either 80 or 160 \( \mu g/kg \) of PTH compared with vehicle controls, indicating that PTH administered daily decreased bone resorption in these rats.

\( (+210\% \text{ to } 456\%) \), mineral apposition rate (+141\% \text{ to } 206\%) and bone formation rate (+359\% \text{ to } 962\%) by 15 days of administration (table 3). Linear regression analysis showed that there was a dose-dependent increase in percent labeled perimeter \( (r = .745, P < .001) \) and bone formation rate \( (BFR/BV) (r = .699, P < .001) \) between 40 and 160 \( \mu g/kg \) of PTH treatment by 15 days. No further increase in these bone formation indices was found by 60 days of treatment. Compared with the same dose groups at day 15, percent labeled perimeter and BFR/BV at both 80 and 160 \( \mu g/kg \) decreased significantly at 60 days of treatment, although these parameters were still significantly increased compared with the vehicle controls at 60 days. Osteoclast number/perimeter was significantly decreased at both 15 and 60 days in rats treated with either 80 or 160 \( \mu g/kg \) of PTH compared with vehicle controls, indicating that PTH administered daily decreased bone resorption in these rats.
increased bone formation and bone mass in the immobilized, osteopenic proximal tibial metaphysis. In our study, bone formation rate (BFR/BV) was increased by 6- to 10-fold at 15 days. At 60 days, treatment with PTH increased bone formation rate, but the rate had decreased to a 2-fold increase, as was seen by Ma and co-workers after 75 days of dosing (Ma et al., 1995). This result indicated that the increased bone formation activity by PTH occurred by 15 days, but persisted over a longer period of time as the bone mass increased from day 15 to day 60. Trabecular bone volume was continuously increased at all levels of PTH treatment by 60 days. In this study, osteoclast number/periosteum was decreased with PTH treatment at 80 and 160 μg/kg/d at both 15 and 60 days, suggesting that resorption is depressed in these aged female rats. Trabecular number and thickness were increased by PTH treatment, which is consistent with bone mass increases shown by DEXA.

There was a significant dose-dependent increase in mineral apposition rate and bone formation rate at 15 days. At 60 days of treatment, there was a significant increase in formation, but it was not dose dependent. No dose-dependent response was found in bone mineral density at all sites measured, at either timepoint.

If these data can be confirmed in human clinical studies, then PTH may be an effective agent to restore bone to the entire skeleton. However, if it only increases BMD of the spine and not the hip, as shown by Mitlak et al. (1994) and Lindsay et al. (1997), then PTH as a therapeutic agent would have significantly less importance as an option in osteoporotic patients. It is expected that an anabolic agent should be able to restore bone in severe osteopenic states, however, as shown by Qi et al. (1995) PTH is ineffective in this model in animals. Therefore, the usefulness of this agent in severe osteoporotic patients needs further study. In conclusion, we have shown that aged female rats treated with PTH (1–34) have an increased whole body bone mass by stimulating bone formation and inhibiting bone resorption.

References


Send reprint requests to: Hollis Simmons, Osteoporosis Group, Central Research Division, Pfizer, Inc., Groton, CT 06340. E-mail: hollis_a_simmons@pfizer.com