Short-term continuous infusion of human parathyroid hormone 1–34 fragment is catabolic with decreased trabecular connectivity density accompanied by hypercalcemia in C57BL/J6 mice

A Iida-Klein1,4, S Shou Lu1, R Kapadia3, M Burkhart3, A Moreno1, D W Dempster1,4 and R Lindsay2,5

1Regional Bone and 2Clinical Research Centers, Helen Hayes Hospital, West Haverstraw, New York, USA
3Scanco USA Inc., Wayne, Pennsylvania, USA
Departments of 4Clinical Pathology and 5Medicine, Columbia University, College of Physicians and Surgeons, New York, New York, USA

Abstract

Parathyroid hormone (PTH) stimulates bone resorption as well as bone formation in vivo and in organ culture. The catabolic actions of PTH have been recognized in patients with hyperparathyroidism, or with acute infusion of the N-terminal 1–34 fragment of human PTH (hPTH1–34). Whereas the anabolic actions of daily injection with PTH have been well studied in both humans and mice, the catabolic actions of PTH on murine bone remain to be defined. To do this we sought to create a model with short-term, sustained hyperparathyroidism using osmotic infusion pumps. We treated 10-week-old female C57BL/J6 mice with continuous infusion of hPTH1–34 (8·1 pmol/0·25 µl per h, equivalent to 40 µg/kg per day) or vehicle for 2 weeks, using Alzet osmotic pumps. Bone mineral density (BMD), serum total calcium, hPTH1–34, mouse intact PTH (mPTH1–84), osteocalcin and mouse tartrate-resistant acid phosphatase (mTRAP) activity, and microarchitectural variables of the distal femur were measured. Separately, we compared the effects of intermittent daily injection of hPTH1–34 (40 µg/kg per day) with continuous infusion of hPTH1–34 on BMD and bone markers. Exogenous hPTH1–34 was detected only in the PTH-infused mice. Both intermittent and continuous treatment with hPTH1–34 markedly suppressed endogenous mPTH1–84, but only the latter induced hypercalcemia. Daily PTH injection significantly increased both serum osteocalcin and mTRAP, while continuous PTH infusion showed a strong trend to stimulate mTRAP, with a slight but non-significant increase in osteocalcin. There were significant differences in BMD at all sites between animals treated with the same daily dose of intermittent and continuous hPTH1–34. Micro-computed tomography (µCT) analysis of the distal femurs revealed that hPTH1–34 infusion significantly decreased trabecular connectivity density (P<0.05). Thus, the murine bone response to continuous PTH infusion was quite different from that seen with daily PTH injection. Short-term infusion of hPTH1–34 appears to be a good model to study the mechanisms underlying the catabolic action of PTH in mice.


Introduction

In humans, continuous elevation of serum parathyroid hormone (PTH) level, as in patients with primary hyperparathyroidism, is catabolic to bone (Cosman et al. 1991, Grey et al. 1996, Bilezikian & Silverberg 2004, Bilezikian et al. 2005). On the other hand, intermittent daily injection with the N-terminal 1–34 fragment of human PTH (hPTH1–34) is an approved anabolic therapy for the treatment of osteoporosis (Reeve et al. 1980, Lindsay et al. 1997, Duan et al. 1999, Cosman et al. 2001, Chen et al. 2003, Finkelstein et al. 2003, Fukata et al. 2004, Dobning 2004). Infusion of hPTH1–34 (0·55 U/kg per h or 25 µg/24 h) to normal and osteoporotic women suppressed endogenous intact human PTH (hPTH1–84) secretion, decreased bone formation and increased bone-resorption markers (Cosman et al. 1991). Chen et al. (2003) compared bone mass and structure among patients with hyper- and hypoparathyroidism and control subjects by dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT), and demonstrated that bone mineral density (BMD) was highest in patients with hypoparathyroidism, intermediate in controls, and lowest in patients with hyperparathyroidism, confirming that an excess of endogenous PTH is catabolic to bone. The cellular and molecular
mechanisms underlying the dual actions of PTH remain to be clearly defined.

Various studies have been undertaken in rats and mice to attempt to create animal models exhibiting the dual actions of PTH. Regarding the anabolic action, it has been well established that intermittent treatment with PTH, both N-terminal 1–34 fragment and intact 1–84 peptide, is anabolic in a range of species including mice (Jilka et al. 1999, Alexander et al. 2001, Andersson et al. 2001, Iida-Klein et al. 2002, Fukata et al. 2004, Rhee et al. 2004, Seebach et al. 2004). However, there are very few studies that demonstrate the catabolic effects of continuously elevated PTH on bone in mice. In ovariectomized rats, Shen et al. (2000) demonstrated that estrogen deficiency induced marked bone loss, and that a 4-week-infusion of rat PTH1–34 induced an additional, significant bone loss in the femur. In the same study, histomorphometric analysis revealed that PTH infusion further increased bone turnover, partially restored bone volume as a percentage of tissue volume and increased cortical porosity in these ovariectomized rats (Zhou et al. 2001). Frolik et al. (2003) have shown that once-daily injection of hPTH1–34 (80 µg/kg per day) increased bone mass in rats, whereas repeated hourly injections over 6 h (6 × (13.3 µg/kg per h)) at a total dose of 80 µg/kg per day PTH decreased bone mass. Imanishi et al. (2001) have created transgenic mice with parathyroid-targeted overexpression of cyclin D1 and demonstrated that these mice exhibited an abnormal relationship between serum calcium and PTH response, similar to that seen in human primary hyperparathyroidism caused by sporadic parathyroid gland hyperplasia (Arnold et al. 2002). Although this transgenic mouse model is an attractive model of hyperparathyroidism, it would also be desirable to develop a model of hyperparathyroidism in wild-type mice that can be produced by simple manipulation or treatment without disruption of genetic structures, allowing studies of the catabolic and anabolic effects of PTH in transgenic mice with various genetic manipulations.

Using 6-week-old female mice, Grey et al. (1999) have shown that a 5-day infusion of hPTH1–84 stimulated interleukin–6 production, which resulted in a significant increase in urinary collagen cross-link levels. This PTH-induced increase in bone resorption was markedly suppressed by interleukin–6 antibody injection and in interleukin–6 knockout transgenic mice, suggesting that interleukin–6 is a mediator of bone-resorbing action of PTH in vivo. Masiukiewicz et al. (2000) demonstrated that ovariectomy increased the catabolic effect of a 5-day hPTH1–84 infusion in mice, and that estrogen treatment prevented this enhanced response to PTH infusion. Few studies in mice have gone beyond 5 days of infusion, and there are no data with regard to the effects of continuous PTH on bone structure or on formation and resorption markers. Therefore, in the present study, we explored the feasibility of delivering PTH continuously by implantation of infusion pumps for up to 14 days and examining various bone measures.

Table 1 Experimental protocol

(a) Experiment 1: pilot studies of 2-week infusion of hPTH1–34

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (weeks)</th>
<th>n</th>
<th>Treatment</th>
<th>Euthanasia (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Control</td>
<td>10</td>
<td>5</td>
<td>Vehicle infusion, 14 days</td>
<td>12</td>
</tr>
<tr>
<td>B. PTH</td>
<td>10</td>
<td>2</td>
<td>(8.1 pmol/0.25 µl per h) × 14 days</td>
<td>12</td>
</tr>
<tr>
<td>Experiment 1b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Control</td>
<td>10</td>
<td>4</td>
<td>Vehicle infusion, 14 days</td>
<td>12</td>
</tr>
<tr>
<td>B. PTH</td>
<td>10</td>
<td>6</td>
<td>(8.1 pmol/0.25 µl per h) × 14 days</td>
<td>12</td>
</tr>
</tbody>
</table>

(b) Experiment 2: comparison between intermittent and continuous hPTH1–34 treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (weeks)</th>
<th>n</th>
<th>Treatment</th>
<th>Euthanasia (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Basal</td>
<td>10</td>
<td>6</td>
<td>Vehicle infusion, 14 days</td>
<td>10</td>
</tr>
<tr>
<td>D. Control injection</td>
<td>10</td>
<td>6</td>
<td>Vehicle infusion, 14 days</td>
<td>12</td>
</tr>
<tr>
<td>E. PTH injection</td>
<td>10</td>
<td>6</td>
<td>hPTH1–34 (40 µg/kg per day) × 14 days</td>
<td>12</td>
</tr>
<tr>
<td>F. Control infusion</td>
<td>10</td>
<td>6</td>
<td>Vehicle infusion, 14 days</td>
<td>12</td>
</tr>
<tr>
<td>G. PTH infusion</td>
<td>10</td>
<td>6</td>
<td>(8.1 pmol/0.25 µl per h) × 14 days</td>
<td>12</td>
</tr>
</tbody>
</table>

One of the PTH infusion mice in experiment 1b showed no detectable hPTH1–34 levels, so all data from this animal were excluded from analysis.
and comparing the effects with those of daily subcutaneous injections of hPTH1–34.

**Materials and Methods**

**Materials**
hPTH1–34 was purchased from Bachem (Torrance, CA, USA). A diagnostic kit for total serum calcium measurement, ketamine, xylazine and all biochemical reagents were purchased from Sigma Chemicals, Co. (St Louis, MO, USA).

**Animals**
The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Helen Hayes Hospital, West Haverstraw, NY, USA. Virgin female C57BL/J6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and stabilized at Helen Hayes Hospital for 2 weeks before the implantation of Alzet pumps (DURECT Corp., Cupertino, CA, USA). Alzet pumps were aseptically filled with appropriate amounts of hPTH1–34 (8·1 pmol/0·25 µl per h, or 40 µg/kg per day equivalent) or vehicle (equivalent volume of 10 mM acetic acid in sterile PBS, pH 7·42) 1 day prior to implantation and stored at 4°C until use. According to the manufacturer’s guidelines, the pumps were implanted subcutaneously into the back of the neck of mice under anesthesia (ketamine 100 mg/kg/xylazine 3 mg/kg) and the incision closed. All procedures were performed aseptically. After implantation of the pumps, animals were individually housed, given free access to water and fed a standard diet (Purina Mills, St Louis, MO, USA) in a room maintained at 22°C with 60–75% humidity on a 12-h light/12-h dark cycle, and carefully monitored for any signs of stress, bleeding, pain or abnormal behavior.

Two independent experiments were performed (Table 1). All animals were randomly divided into groups 2 days prior to implantation of the pumps. Experiment 1 was a feasibility experiment to determine whether a 2-week infusion was possible in mice and to compare the murine response to hPTH1–34 with control, vehicle-infused animals. In our pilot trial (Exp1a) we found that in vivo BMD measurement immediately before implantation of the pumps caused marked distress, resulting in loss of three out of five mice with PTH infusion within 2 h of surgical operations. Thus, we performed an additional experiment (Exp1b; control n=4, PTH n=6). BMD was measured 1 day prior to implantation of pumps. Data from experiments 1a and 1b were combined as experiment 1 (control n=9, PTH n=8), as there were no significant within-group differences in BMD and serum biochemical markers between the animals in experiments 1a and 1b.

At 2 weeks, bone structure and serum levels of total calcium, hPTH1–34 and endogenous mouse intact PTH (mPTH1–84) were measured. The right femurs in Exp1b (control n=4, PTH n=6) were excised, cleaned, partially embedded in methacrylate and sent to Scanco USA (Wayne, PA, USA) for further analysis of bone microarchitecture and structure assessed by micro-computed tomography (μCT).

Experiment 2 was a short-term comparison of the effects of continuous PTH infusion (n=6) compared with intermittent PTH daily injection (n=6) with BMD and biochemical markers as the outcomes. Baseline BMD was measured 1 day prior to the initiation of treatment.
At euthanasia, blood was collected by cardiac puncture, and serum stored at −80°C for biochemical assays. For the intermittent treatment, animals were euthanized approximately 20–24 h after the last PTH injection.

**BMD measurement by PIXImus**

BMD was measured weekly and 1 day prior to initiation of treatment by DXA using PIXImus (GE Lunar Corp., Madison, WI, USA) as previously described (Iida-Klein et al. 2002, 2003). Briefly, mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (3 mg/kg), placed prone on the platform of the PIXImus and BMD measured with mouse-specific software (PIXImus, version 1·47). The machine was calibrated daily with the manufacturer’s phantom. The scans were analyzed, and the regions of interest defined as previously described (Iida-Klein et al. 2002, 2003).

**µCT analysis**

The distal femurs were scanned by Scanco USA using the Desktop Cone-Beam µCT Scanner (µCT40; Scanco USA), with 10 µm isotropic pixel size, 70 kV energy, 114 µA intensity and an integration time of 300 ms. The volume of interest with a length of 1·70 mm was placed in an axial direction in the secondary spongiosa of the distal femurs, just above the growth plate. Pixel dimensions were set at 2048 × 2048 × 170 longitudinally to expose the bone marrow (Laib et al. 2000, 2001). Measured variables their abbreviations were as defined by Parfitt et al. (1987).

**Biochemical assays**

Serum total calcium was determined in duplicate by colorimetric reaction of calcium ion with o-cresolphthalein complexone in the presence of 8-hydroxyquinoline, and the optical density of this colored compound was measured at 575 nm (Kang et al. 2004). Serum levels of infused hPTH1–34 and endogenous mPTH1–84 were determined using ELISA kits (Immutopics, San Clemente, CA, USA) according to the manufacturer’s instructions. There was no cross-reactivity between the antibodies against hPTH1–34 and mPTH1–84 used in these ELISA kits. Mouse tartrate-resistant acid phosphatase (mTRAP; Immunodiagnostic System, Phoenix, AZ, USA) ELISA assay was performed according to the manufacturer’s instructions. For mouse osteocalcin immunoradiometric assay (Immutopics), 10 µl of serum was diluted 11 times with buffer, and osteocalcin levels were measured using the diluted samples according to the manufacturer’s instructions. Serum creatinine and urea nitrogen (BUN; Thermo Electron Clinical Chemistry, Louisville, CO, USA) were spectrophotometrically measured at 500 and 340 nm, respectively. Mean intra-assay coefficients of variation were 5·6, 3·5, 4·7, 2·4 and 5·0% for calcium, hPTH1–34, mPTH1–84, osteocalcin and mTRAP, respectively.

**Statistical analysis**

All values for BMD and biochemical marker measurements represent means ± S.E.M. Significant differences between groups were determined by one-way (experiment 1) or two-way (experiment 2) ANOVA (Duncan) using SAS (version 9·1; Cary, NC, USA).

**Results**

At the end of 2 weeks, there was no significant difference in body weight between groups of animals with vehicle and PTH infusion in all experiments: at 0 weeks, the body weight was 18·27 ± 0·25 and 18·51 ± 0·26 g for animals with vehicle (n=15) and PTH (n=14) infusion (P=0·518), respectively, and at 2 weeks this increased to

<table>
<thead>
<tr>
<th>Sites</th>
<th>Time of BMD measurement (mg/cm²)</th>
<th>0 weeks</th>
<th>1 week</th>
<th>2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>Control infusion</td>
<td>56·83 ± 0·71</td>
<td>54·88 ± 0·74</td>
<td>55·38 ± 1·00</td>
</tr>
<tr>
<td></td>
<td>PTH infusion</td>
<td>57·29 ± 0·44</td>
<td>53·67 ± 0·51</td>
<td>55·97 ± 0·50</td>
</tr>
<tr>
<td>Tibia</td>
<td>Control infusion</td>
<td>41·26 ± 0·55</td>
<td>39·08 ± 0·33</td>
<td>40·75 ± 0·80</td>
</tr>
<tr>
<td></td>
<td>PTH infusion</td>
<td>41·31 ± 0·48</td>
<td>39·93 ± 0·23</td>
<td>40·83 ± 0·71</td>
</tr>
<tr>
<td>Lumbar vertebrae 4–5</td>
<td>Control infusion</td>
<td>51·39 ± 0·97</td>
<td>49·77 ± 0·78</td>
<td>49·84 ± 1·46</td>
</tr>
<tr>
<td></td>
<td>PTH infusion</td>
<td>53·80 ± 0·58</td>
<td>49·80 ± 1·04</td>
<td>49·33 ± 1·25</td>
</tr>
</tbody>
</table>

Animals were treated continuously with vehicle or hPTH1–34 as described in the Materials and Methods section. (n=9 for control vehicle infusion, n=7 for PTH infusion).
20.36 ± 0.32 and 20.1 ± 0.24 g for vehicle and PTH infusion (P=0.511), respectively. No animals showed abnormal movements or signs of stress or discomfort at any point in any experiment.

**Experiment 1**

Figure 1 shows the serum levels of hPTH1–34 (Fig. 1A) and mPTH1–84 (Fig. 1B) after a 2-week infusion. As expected, no hPTH1–34 was detected in mice infused with vehicle, whereas PTH–infused animals exhibited significant circulating levels of hPTH1–34 (79.79–20.31 pM, P<0.02; Fig. 1A). Infusion of hPTH1–34 significantly suppressed endogenous intact mPTH1–84 from 2.40 ± 0.55 pM (control) to 0.73 ± 0.25 pM (P<0.02; Fig. 1B), and significantly increased total serum calcium levels from 1.95 to 2.64 mM (P<0.005; Fig. 1C). One animal that did not show a detectable level of hPTH1–34 after implantation of the pump containing hPTH1–34 was excluded from all analyses.

There was no significant difference in BMD between control and hPTH1–34-infused mice at 2 weeks at all sites (Table 2). However, using µCT, femurs from mice infused with hPTH1–34 exhibited thinner trabeculae and greater trabecular spacing compared with control mice (Fig. 2). Moreover, hPTH1–34 infusion decreased trabecular connectivity density by 40% (P<0.05; Table 3).

### Table 3 Effects of hPTH1–34 infusion on bone structure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PTH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vox BV/TV (%)</td>
<td>8.2 ± 0.7</td>
<td>6.4 ± 0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Connectivity density (1/mm³)</td>
<td>92.64 ± 12.14</td>
<td>55.56 ± 8.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TRI-SMI (structure model index)</td>
<td>2.67 ± 0.14</td>
<td>3.06 ± 0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>TRI-bone surface (mm³)</td>
<td>13.55 ± 1.64</td>
<td>10.52 ± 0.53</td>
<td>0.16</td>
</tr>
<tr>
<td>TRI-BV/TV (%)</td>
<td>7.7 ± 0.7</td>
<td>5.9 ± 0.4</td>
<td>0.09</td>
</tr>
<tr>
<td>TRI-Tb.N (1/mm)</td>
<td>2.66 ± 0.24</td>
<td>2.19 ± 0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>TRI-Tb.Th (µm)</td>
<td>28.8 ± 0.7</td>
<td>26.7 ± 0.6</td>
<td>0.08</td>
</tr>
<tr>
<td>TRI-Tb.Sp (µm)</td>
<td>358 ± 39</td>
<td>433 ± 20</td>
<td>0.16</td>
</tr>
<tr>
<td>DT-Tb.N (1/mm)</td>
<td>4.27 ± 0.17</td>
<td>4.23 ± 0.10</td>
<td>0.86</td>
</tr>
<tr>
<td>DT-Tb.Th (µm)</td>
<td>38.6 ± 1.2</td>
<td>37.5 ± 0.6</td>
<td>0.48</td>
</tr>
<tr>
<td>DT-Tb.Sp (µm)</td>
<td>234 ± 11</td>
<td>236 ± 6</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Vox, Based on counting voxels; SMI, 0 for parallel plates, 3 for cylindrical rods; TRI, based on triangulation of surface (one more interpolation step), assuming that bone is made of parallel plates, two-dimensional histomorphometry; DT, based on distance transformation (filling structure with spheres), without plate model assumption, three-dimensional histomorphometry; BV/TV, bone volume as a percentage of tissue volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation.
There was a trend towards a decrease in trabecular volume, trabecular number and trabecular thickness in mice infused with hPTH1–34 (Table 3).

Experiment 2

As seen in experiment 1, exogenously administered hPTH1–34 was detected only in mice with hPTH1–34 infusion but not in mice with vehicle infusion or daily injection of hPTH1–34 (Fig. 3A). Both infusion and daily injection of PTH suppressed endogenous mPTH1–84 compared with controls (Fig. 3B). Moreover, continuous infusion, but not daily injection of hPTH1–34, produced hypercalcemia (Fig. 3C). Serum creatinine and BUN levels were less than 9 µM and 1·8 mM, respectively, in all groups and there was so significant difference among groups.

The serum osteocalcin level was increased by 43% \((P<0·005)\) with intermittent hPTH1–34 treatment, but not with continuous hPTH1–34 treatment (9% increase, \(P=0·371\); Fig. 4A). The resorption marker, mTRAP, was increased by 223% \((P<0·005)\) and 214% \((P=0·066)\) in mice on intermittent and continuous hPTH1–34 treatment, respectively (Fig. 4B).

There were significant differences in BMD between animals treated with hPTH1–34 in the continuous and intermittent modes of administration at all sites measured at 1 and 2 weeks (Fig. 5). Moreover, there was a slight but significant decrease in femoral BMD in PTH-infused mice compared with that in vehicle-infused mice (Fig. 5B). However, there was no significant difference in BMD between vehicle- and PTH-infused mice at the other skeletal sites.

Discussion

This is the first report demonstrating that a 2-week continuous infusion of hPTH1–34 exerts a catabolic effect...
on mouse bone with a significant decrease in trabecular connectivity density. Moreover, we showed that whereas both infusion and daily injection of exogenous hPTH1–34 significantly suppressed endogenous mPTH1–84, only PTH infusion induced hypercalcemia at 2 weeks of treatment. Both infusion and daily injection of PTH increased a bone-resorption marker to a similar extent, but only the latter significantly increased a formation marker. We also demonstrated that there was a significant difference in BMD at all sites between continuous infusion and daily injection of PTH.

Intact serum PTH1–84 levels in normal healthy humans range from 1 to 4 pM (mean, 2.4 pM), and increase to 3–20 pM (mean, 8.4 pM) in patients with primary hyperparathyroidism (Rajala et al. 1991), and generally parallel increased serum calcium. In patients and animals with kidney failure, secondary hyperparathyroidism develops and serum PTH levels may exceed 100 pM (Bover et al. 1994, de Francisco 2004). Transgenic mice with parathyroid-targeted overexpression of cyclin D1 exhibit mild hyperparathyroidism, with PTH ranging between 13 and 16 pM – 3–4-fold higher than their age-matched wild-type littermates (2–4 pM) – and a 20–30% increase in total serum calcium (Imanishi et al. 2001, Arnold et al. 2002). However, it takes 12–13 months for this model to exhibit significant differences in biochemical phenotypes between transgenic and wild-type mice (Imanishi et al. 2001). In our current infusion model, intact mPTH1–84 was significantly suppressed, although serum bioactive PTH levels (total PTH = infused hPTH1–34 + endogenous intact mPTH1–84) were >100 pM in 7 out of 14 PTH-infused mice. However, serum creatinine and BUN were within the normal range in all groups at the completion of infusion, confirming normal renal function. No animals exhibited abnormal movements or signs of stress or discomfort, and there was no significant difference in body

Figure 5 Comparison of the effects of continuous versus intermittent treatment with hPTH1–34 on BMD. Animals were treated either intermittently or continuously with hPTH1–34 and BMD was measured weekly by PIXImus™ as described in the Materials and Methods section. Definition of the regions of interest was described elsewhere (Iida-Klein et al. 2002, 2003). Each scan was analyzed twice and averaged (n=6 for each group). LV4–5, lumbar vertebrae 4–5.
weight between animals treated with vehicle and PTH
infusion, with all animals gaining approximately 1·5–2 g
during 2 weeks of treatment. Mice with parathyroid-
specific cyclin D1 overexpression with mild hyperpara-
thyroidism and hypercalcemia did not exhibit any abnor-
mal phenotype, except changes in calcium balance and
biochemical status, and lived longer than 2 years (Imanishi
et al. 2001, Arnold et al. 2002). In contrast to this model of
chronic, mild hyperparathyroidism, our model may be
more useful to define the mechanisms underlying the dual
actions of PTH in the short-term and can be applied to a
variety of transgenic mice with different phenotypes.

Mice receiving continuous hPTH1–34 infusion exhib-
ted an increase in mTRAP, a bone-resorption marker,
that was similar to those receiving daily hPTH1–34
injection (214 versus 238% increase), but failed to show
a significant increase in a bone-formation marker, osteo-
calcin. Thus, PTH infusion-induced hypercalcemia may
be primarily due to stimulated resorption without any
disturbance of kidney function. Since it has been consid-
ered that osteocalcin is a later formation marker, use of
other, earlier formation markers in a short-term study
may be more appropriate. Moreover, we only examined
the biochemical markers at the endpoint of the study.
Development of better assay systems that can measure
bone-formation and -resorption markers in smaller blood
samples from live mice at earlier time points will be helpful
for future studies.

In the current study, endogenous, intact mPTH1–84
was significantly reduced by both intermittent and con-
tinuous PTH treatment. This is consistent with observa-
tions in human studies (Cosman et al. 1991, Lindsay
et al. 1993).

The rodent skeletal response to the catabolic action of
PTH is very sensitive to estrogen status in both rats (Shen
et al. 1993, Zhou et al. 2001) and mice (Masiukiewicz et
al. 2000). In the present study, in which the mice were not
ovariectomized, it is possible that endogenous estrogen
may have blocked the catabolic effects of PTH infusion on
bone mass. This may be one explanation for why no
clear catabolic effect of PTH infusion was observed by
densitometry in the PTH-infused mice, although µCT
analysis revealed a significantly reduced trabecular connec-
tivity density. Based on these observations, we speculate
that BMD measurement by DXA may be too insensitive
to detect the mild, early catabolic effects produced by
continuous PTH treatment, although it can easily detect
the more dramatic, anabolic effects of intermittent PTH
treatment.

Further investigation is required to establish PTH
infusion as a murine model of hyperparathyroidism,
including the temporal changes in serum calcium and
bone markers, dose dependence, static and dynamic
histomorphometry and vitamin D status. However, the
current study clearly demonstrated the feasibility of this
approach, and indicated that short-term infusion with
hPTH1–34 might hold significant promise as a murine
model to study the cellular and molecular mechanisms of
the catabolic action of PTH in animals with relatively
normal physiology and without complicated genetic
manipulation.

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NS Pharma, and the Helen Hayes Hospital Foundation.
The authors declare that there is no conflict of interest that
would prejudice the impartiality of this scientific work.

References

Alexander JM, Bab I, Fish S, Müller R, Uchiyama T, Gronowicz G,
Nalhounou M, Zhao Q, White DW, Choret M et al. 2001 Human
parathyroid hormone 1–34 reverses bone loss in ovariectomized

Andersson N, Lindberg MK, Ohlsson C, Andersson K & Ryberg B
2001 Repeated in vivo determination of bone mineral density
during parathyroid hormone treatment in ovariectomized mice.
Journal of Endocrinology 170 529–537.

Arnold A, Shattuck TM, Mallya SM, Krebs LJ, Costa J, Gallagher J,
Wild Y & Saucier K 2002 Molecular pathogenesis of primary
2 N30–N36.

primary hyperparathyroidism. New England Journal of Medicine
350 1746–1751.

Bilezki JP, Brandi ML, Rubin M & Silverberg SJ 2005 Primary
hyperparathyroidism: new concepts in clinical, densitometric and

Felsenfeld AF 1994 The calcemic response to PTH in the rat: effect
of elevated PTH levels and uremia. Kidney International
46 310–317.

Chen Q, Kaji H, Iu MF, Nomura R, Sowa H, Yamauchi M,
Tsukamoto T, Sugimoto T & Chihara K 2003 Effects of an excess
and a deficiency of endogenous parathyroid hormone on volumetric
bone mineral density and bone geometry determined by peripheral
quantitative computed tomography in female subjects. Journal of
Clinical Endocrinology and Metabolism 88 4655–4658.

Cosman F, Shen V, Herrington B & Lindsay R 1991 Response of the
parathyroid gland to infusion of human parathyroid hormone-(1–34)
[PTH-(1–34)]: demonstration of suppression of endogenous
secretion using immunoradiometric intact PTH-(1–84) assay. Journal
of Clinical Endocrinology and Metabolism 73 1345–1351.

Cosman F, Nieves J, Woelfert L, Formica C, Gordon S, Shen V &
Lindsay R 2001 Parathyroid hormone added to established hormone
therapy: effects on vertebral fracture and maintenance of bone mass
after PTH withdrawal. Journal of Bone and Mineral Research
16 925–931.

Dobng H 2004 A review of teriparatide and its clinical efficacy in the
treatment of osteoporosis. Expert Opinion on Pharmacotherapy
5 1153–1162.

Duan Y, De Luca V & Seeman E 1999 Parathyroid hormone
deficiency and excess: similar effects on trabecular bone but
differing effects on cortical bone. Journal of Clinical Endocrinology
and Metabolism 84 718–722.


Shen V, Dempster DW, Birchman R, Xu R & Lindsay R 1993 Loss of cancellous bone mass and connectivity in ovariectomized rats can be restored by combined treatment with parathyroid hormone and estradiol. *Journal of Clinical Investigation* **91** 2479–2487.
