

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

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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.

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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, Dobnig 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

Table 1 Experimental protocol

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

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

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

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

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.

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). Frolík *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,

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 μ l per h, or 40 μ g/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.

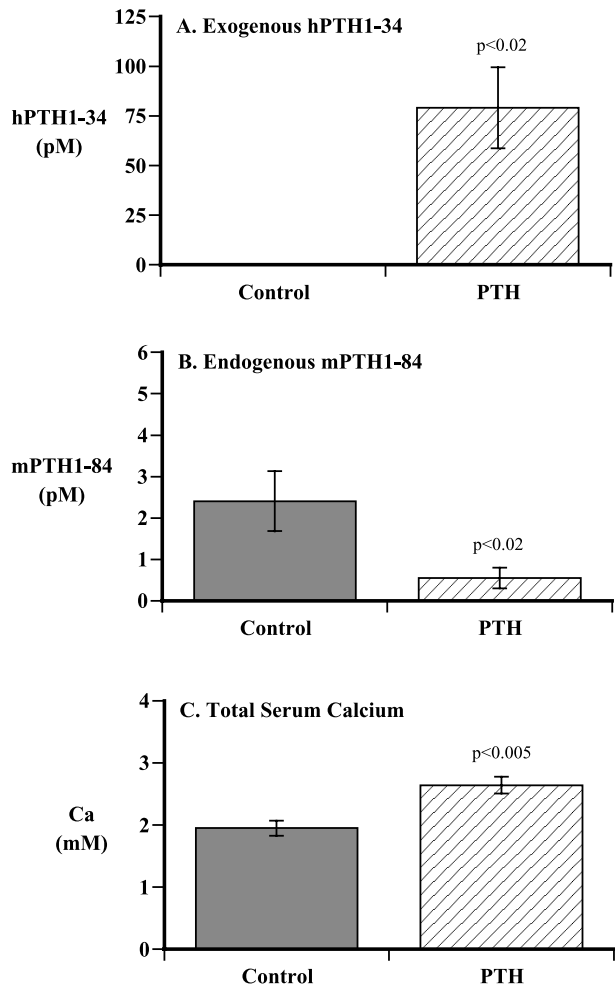


Figure 1 Effects of a 2-week infusion with hPTH1–34 on serum hPTH1–34 (A), mPTH1–84 (B) and total calcium (C) in female C57BL/J6 mice. Animals were continuously treated with hPTH1–34 (8.1 pmol/0.25 μ l per h) using Alzet pumps and blood collected for biochemical assays as described in the Materials and Methods section. All samples were assayed in duplicate, and averaged. Values are means \pm S.E.M. ($n=9$ for control vehicle infusion, $n=8$ for PTH infusion).

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 micro-architecture and structure assessed by micro-computed tomography (μ CT).

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.

Table 2 Effect of a 2-week infusion of hPTH1–34 on BMD in C57BL/6 mice

Sites	Time of BMD measurement (mg/cm ²)		
	0 weeks	1 week	2 weeks
Femur			
Control infusion	56.83 ± 0.71	54.88 ± 0.74	55.38 ± 1.00
PTH infusion	57.29 ± 0.44	53.67 ± 0.51	55.97 ± 0.50
Tibia			
Control infusion	41.26 ± 0.55	39.08 ± 0.33	40.75 ± 0.80
PTH infusion	41.31 ± 0.48	39.93 ± 0.23	40.83 ± 0.71
Lumbar vertebrae 4–5			
Control infusion	51.39 ± 0.97	49.77 ± 0.78	49.84 ± 1.46
PTH infusion	53.80 ± 0.58	49.80 ± 1.04	49.33 ± 1.25

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).

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 ($\mu\text{CT}40$; 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 $2048 \times 2048 \times 170$ 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 \pm 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

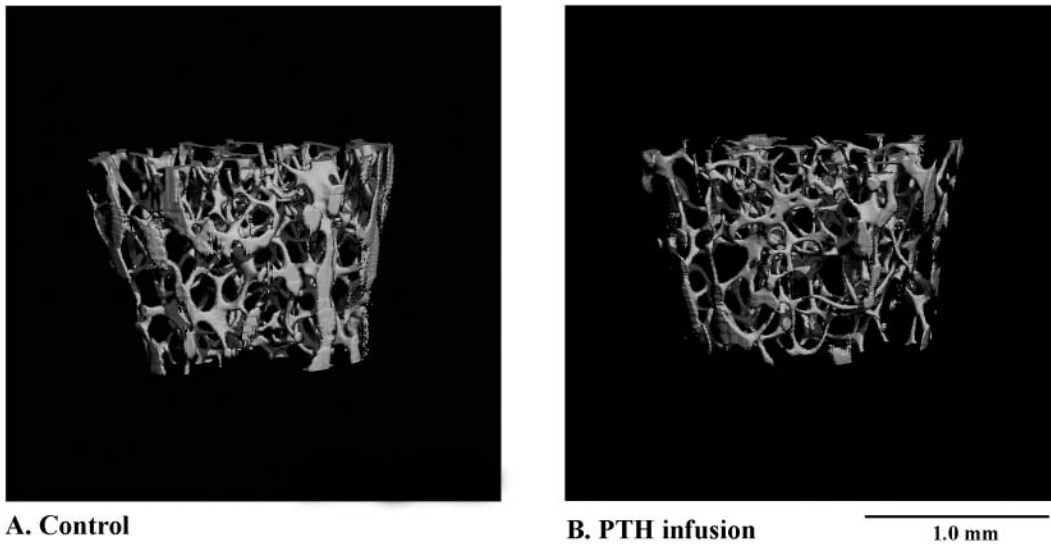


Figure 2 Trabecular bone microarchitecture by μ CT analysis in the distal femur from vehicle-infused (A) and hPTH1–34-infused (B) mice.

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 ($79.79\text{--}20.31$ 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 μ CT, 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

	Control	PTH	P value
Vox BV/TV (%)	8.2 ± 0.7	6.4 ± 0.4	0.1
Connectivity density ($1/\text{mm}^3$)	92.64 ± 12.14	55.56 ± 8.08	<0.05
TRI-SMI (structure model index)	2.67 ± 0.14	3.06 ± 0.06	0.06
TRI-bone surface (mm^2)	13.55 ± 1.64	10.52 ± 0.53	0.16
TRI-BV/TV (%)	7.7 ± 0.7	5.9 ± 0.4	0.09
TRI-Tb.N (1/mm)	2.66 ± 0.24	2.19 ± 0.09	0.15
TRI-Tb.Th (μm)	28.8 ± 0.7	26.7 ± 0.6	0.08
TRI-Tb.Sp (μm)	358 ± 39	433 ± 20	0.16
DT-Tb.N (1/mm)	4.27 ± 0.17	4.23 ± 0.10	0.86
DT-Tb.Th (μm)	38.6 ± 1.2	37.5 ± 0.6	0.48
DT-Tb.Sp (μm)	234 ± 11	236 ± 6	0.90

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.

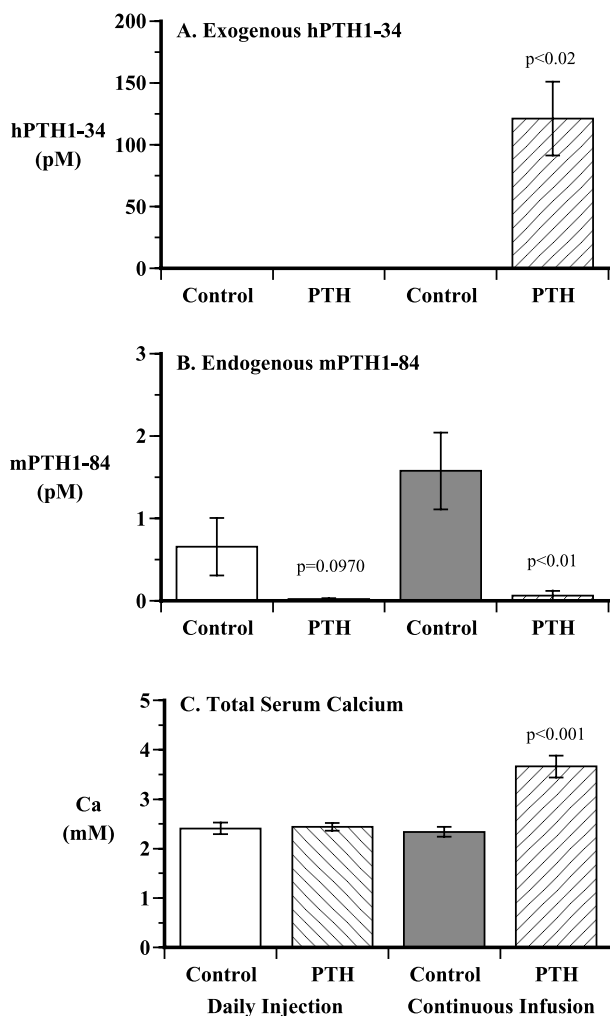


Figure 3 Comparison of the effects of continuous and intermittent treatment with hPTH1–34 on hPTH1–34 (A), mPTH1–84 (B) and total serum calcium levels (C). Animals were treated and blood collected for biochemical assays as described in the Materials and Methods section. Values are means \pm S.E.M. from six mice. Serum levels of hPTH1–34, mPTH1–84 and total calcium of basal mice were 0 pM, 1.71 ± 0.21 pM and 2.37 ± 0.11 mM, respectively, and were not significantly different from the values of vehicle injection or infusion.

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

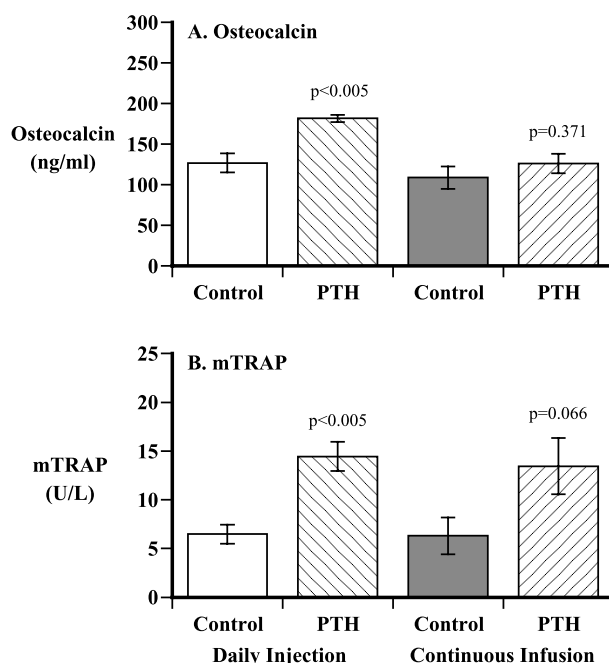


Figure 4 Comparison of the effects of continuous and intermittent treatment with hPTH1–34 on bone-formation (A) and -resorption markers (B). Animals were treated and blood collected for biochemical assays as described in the Materials and Methods section. Values are means \pm S.E.M. from six mice.

infusion, but not daily injection of hPTH1–34, produced hypercalcemia (Fig. 3C). Serum creatinine and BUN levels were less than $9 \mu\text{M}$ and 1.8 mM , respectively, in all groups and there was no 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

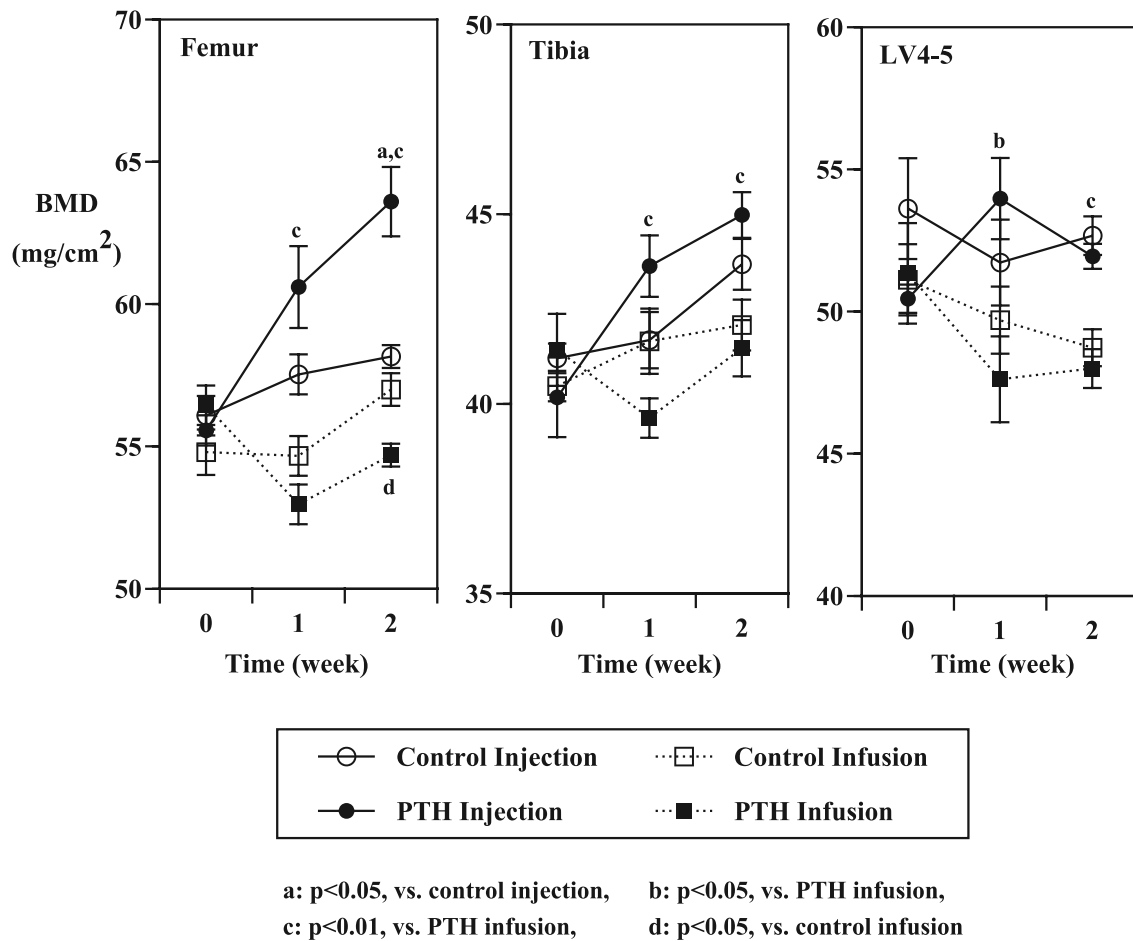


Figure 5 Comparison of the effects of continuous versus intermittent treatment with hPTH1–34 on BMD. Animals were treated either intermittently or continuously with hPTH1–34 and BMD was measured weekly by PIXImus™ as described in the Materials and Methods section. Definition of the regions of interest 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.

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

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 hyperparathyroidism and hypercalcemia did not exhibit any abnormal 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 exhibited 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, osteocalcin. Thus, PTH infusion-induced hypercalcemia may be primarily due to stimulated resorption without any disturbance of kidney function. Since it has been considered 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 continuous PTH treatment. This is consistent with observations 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 μ CT analysis revealed a significantly reduced trabecular connectivity 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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