Parathyroid Hormone-Related Protein for the Treatment of Postmenopausal Osteoporosis: Defining the Maximal Tolerable Dose

Mara J. Horwitz, Mary Beth Tedesco, Adolfo Garcia-Ocaña, Susan M. Sereika, Linda Prebehala, Alessandro Bisello, Bruce W. Hollis, Caren M. Gundberg, and Andrew F. Stewart

Division of Endocrinology and Metabolism (M.J.H., M.B.T., A.G.-O., L.P., A.F.S.) and Department of Pharmacology and Chemical Biology (A.B.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213; The Graduate School of Public Health (S.M.S.), University of Pittsburgh, Pittsburgh Pennsylvania 15261; Department of Pediatrics (B.W.H.), The Medical University of South Carolina, Charleston, South Carolina 29425; and Department of Orthopedics (C.M.G.), Yale University School of Medicine, New Haven, Connecticut 06520

Context: PTH is the only approved skeletal anabolic agent for the treatment of human osteoporosis. Unlike PTH, which is a mixed anabolic and catabolic agent, PTHrP displays features suggesting that it may be a pure anabolic agent when intermittently administered. The full dose range of PTHrP is unknown.

Objectives: The primary objective of the study was to define the complete therapeutic window and dose-limiting toxicities of PTHrP. The secondary objective was to determine whether PTHrP retains a pure anabolic profile at the highest usable doses.

Design: This was a single-blinded, two-part, dose-escalating clinical trial.

Setting: The study was conducted in a university academic setting.

Patients or Other Participants: Participants included 41 healthy postmenopausal women between the ages of 45 and 75 yr.

Intervention: Interventions included PTHrP(1-36) or placebo in a dose-escalating design for 3 wk.

Main Outcome Measures: Safety measures (hypercalcemia, nausea, vomiting, hemodynamics, flushing, miscellaneous) and bone turnover markers were measured.

Results: Intermittent PTHrP was administered safely and without serious adverse events in subjects receiving 500 and 625 μg/d for 3 wk. Subjects receiving 750 μg/d developed mild hypercalcemia. Bone turnover markers suggested that even at the highest doses, daily sc PTHrP may not activate bone resorption, *i.e.* may be purely anabolic. Interestingly, when hypercalcemia occurred, it may have resulted not from bone resorption but from activation of intestinal calcium absorption by 1,25 dihydroxyvitamin D.

Conclusions: In doses as high as 750 μg/d, in contrast to PTH, intermittently administered PTHrP appears to act as a pure skeletal anabolic agent. Surprisingly, PTHrP in the high doses studied activates 1,25 dihydroxyvitamin D production. Dosing information obtained herein can be used to design a longer term head-to-head comparative efficacy trial of PTHrP *vs.* PTH. **(*J Clin Endocrinol Metab* 95: 1279–1287, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-0233 Received February 2, 2009. Accepted December 1, 2009.
First Published Online January 8, 2010

Abbreviations: BMD, Bone mineral density; BP, blood pressure; CTX, carboxy-terminal telopeptide of collagen 1; CV, coefficient of variation; DLT, dose-limiting toxicity; FECa, fasting calcium excretion; NTX, amino-terminal telopeptide of collagen-1; 1,25$(OH)_2$D, 1,25 dihydroxyvitamin D_3; P1NP, amino-terminal telopeptide of procollagen 1.

The paradigm for skeletal anabolic drugs is PTH, which can stimulate osteoblasts to form new bone and increase bone mineral density (BMD) to a greater extent than the antiresorptives (1–7). Optimally, skeletal anabolic agents would reduce fracture risk more than antiresorptives. However, dosing of the PTH family members has been limited by hypercalcemia, nausea, flushing, muscle cramping, and other adverse effects (1–7). The hypercalcemia is associated with increases in osteoclast-driven bone resorption (1–7). Thus, PTH is a mixed skeletal anabolic and catabolic agent.

PTHrP is homologous with PTH but differs in several respects, including being products of separate genes, sequence divergence at the amino acid and nucleotide level, and fundamentally differing normal physiology (8, 9). PTHrP is a normal product of osteoblasts and acts, like PTH, via the common PTH-PTHrP receptor (also called the PTH-1 receptor or PTH1R) to activate osteoblast function and, indirectly, recruit osteoclast precursors (9–13). Elegant studies in mice lacking PTH and/or PTHrP have shown that PTHrP is a naturally occurring skeletal anabolic agent and that PTH cannot complement or replace this natural anabolic function (9–13).

PTHrP (1-36), referred to hereafter as PTHrP, can be administered intermittently safely to healthy young adults and postmenopausal women for periods ranging from 1 d to 3 months in doses ranging from 50 to 2000 μg/d (14–17). In the two prior studies in which formation markers were studied (18, 19), PTHrP led to an increase in markers of bone formation. In the single study of sufficient duration (3 months) to assess changes in BMD (16), daily sc PTHrP at a dose of 400 μg/d increased lumbar spine BMD by approximately 5%, comparing favorably with PTH (4–9). Remarkably in each prior study, PTHrP consistently appeared to differ from PTH in that markers of bone resorption were never activated (15, 16). Moreover, in contrast to studies with PTH, even minor hypercalcemia was not observed (14–17). These observations are consistent with the possibility that intermittently administered PTHrP may be a pure skeletal anabolic agent. They also suggest that doses higher than the 400 μg/d used in the prior 3-month study, if tolerated, might enhance BMD to a greater extent than the 400 μg/d dose and also more than maximally tolerated doses of PTH peptides.

Here we report the maximal tolerable dose and the complete therapeutic window of PTHrP for future, longer-term osteoporosis studies. Surprisingly, even at doses associated with hypercalcemia, bone resorption did not appear to be activated, suggesting that intermittently administered PTHrP may be a pure skeletal anabolic agent. Equally surprisingly, serum 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] increased in response to PTHrP and was accompanied by increases in 24-h urine calcium, raising the possibility that when hypercalcemia occurs with high-dose PTHrP, it may not result from bone resorption but from intestinal hyperabsorption of calcium. These studies lay the foundation for definitive long-term studies comparing the efficacy and safety of PTH and PTHrP as skeletal anabolic agents.

Subjects and Methods

Study subjects

Forty-one healthy Caucasian, Hispanic, or Asian postmenopausal women between the ages of 45 and 75 yr were recruited (Table 1). All provided written informed consent. Because the study was too brief to assess BMD outcomes, osteoporosis was not an inclusion criterion and BMD was not measured. Exclusion criteria were prior treatment with antiresorptives, estrogens, or raloxifene; or PTH family members; recent (1 yr) fractures; vascular, cardiac, hypertension, pulmonary, kidney or liver disease; anemia; or cancer or other illnesses. Subjects with baseline serum calcium values greater than 10.5 mg/dl, 25 hydroxyvitamin D values less than 15 ng/ml, or PTH values greater than 65 pg/ml were excluded. The study was approved in advance by the University of Pittsburgh Institutional Review Board. All subjects provided written informed consent. The trial was registered (with clinical trials.gov, no. NCT00222872).

Study design

This was a single-blinded, two-part, dose-escalation trial. The primary outcome measures were safety measures (hypercalcemia, cardiovascular homeostasis, renal function). Secondary outcome measures were markers of bone turnover and 1,25$(OH)_2$D. The first 20 d of the study were performed on an outpatient basis. All laboratory test results were collected immediately before the daily injection except on d 21. On d 21, subjects were hospitalized for 24 h for more intensive monitoring of safety measures at 6, 12, 18, and 24 h after injection. The first 20 subjects were randomized to receive either placebo (saline, n = 10) or PTHrP (1-36) 500 μg/d (n = 10) as sc injections for 21 d. Thereafter the protocol required progressive dose increments of 125 μg (625, 750, 875, 1000 μg/d, *etc.*) in groups of three subjects until either a dose of 1500 μg/d was reached or until dose-limiting toxicity (DLT) occurred. No subject was studied at more than one dose. DLT was defined as one or more major, or two or more minor, adverse events (AEs). Major AEs included a symptomatic decline in systolic blood pressure (BP) greater than 30 mm Hg; development of hypertension (systolic BP >160 mm Hg) on two occasions; tachycardia greater than 120 bpm; development of a serum calcium of 11.0 mg/dl or greater; or development of a serum phosphorus less than 1.5 mg/dl. Minor criteria were defined as flushing, nausea/vomiting, abdominal cramps, muscle cramps, dizziness/lightheadedness, palpitations, or other minor symptoms. The study was terminated immediately in any subject in whom DLT occurred. If no DLT occurred in a group of three subjects at a given dose, dosing progressed to the next level in a cohort of three subjects. If one major AE or two minor AEs occurred, three additional subjects were allowed to accrue at that dose. If two or more major AEs

TABLE 1. Baseline demographics and biochemical analyses

	Placebo (n = 15)	PTHrP 500 μg/d (n = 10)	PTHrP 625 μg/d (n = 10)	PTHrP 750 μg/d (n = 6)
Age (yr)	58 ± 2.1	57 ± 2.2	55 ± 1.6	60 ± 3.0
Height (cm)	162 ± 1.4	159 ± 2.4	165 ± 2.2	162 ± 1.8
Weight (kg)	66 ± 2.5	64 ± 3.1	68 ± 2.6	70 ± 2.8
BMI	25 ± 0.7	25 ± 1.0	25 ± 1.2	27 ± 0.9
Years after menopause	10 ± 2.7	7 ± 1.9	7 ± 1.5	10 ± 2.5
Baseline calcium intake (mg/d)	1037 ± 194	1047 ± 124	861 ± 95	1316 ± 444
Intact PTH (1-84) (pg/ml)	36.7 ± 4.0	37.1 ± 5.9	43.6 ± 4.3	45.0 ± 6.1
Serum 25 vitamin D (ng/ml)	39 ± 4	29 ± 4	31 ± 3	34 ± 3
Serum 1,25 vitamin D (pg/ml)	38.5 ± 3.9	38.9 ± 3.5	39.2 ± 2.5	42.1 ± 3.9
Serum calcium (mg/dl)	9.3 ± 0.1	9.3 ± 0.1	9.5 ± 0.1	9.8 ± 0.1
Serum creatinine (mg/dl)	0.82 ± 0.04	0.83 ± 0.03	0.80 ± 0.06	0.83 ± 0.03
Fasting FECa (%)	2.1 ± 0.2	3.7 ± 1.0	2.9 ± 0.7	3.7 ± 0.8
Serum osteocalcin (ng/ml)	10.6 ± 0.7	12.83 ± 0.9	10.34 ± 0.5	11.0 ± 1.0
Serum P1NP (μg/liter)	52.7 ± 5.9	68.2 ± 9.6	46.0 ± 4.1	64.0 ± 7.9
Serum BSAP (μg/liter)	15.8 ± 1.2	19.7 ± 2.8[a]	11.8 ± 1.3[a]	14.4 ± 1.7
Serum NTX (nM BCE)	16.3 ± 1.9	17.5 ± 1.5	15.1 ± 1.3	19.9 ± 3.9
Serum CTX (ng/ml)	0.77 ± 0.1	0.92 ± 0.1[b]	0.48 ± 0.1[b]	0.57 ± 0.1

BSAP, Bone-specific alkaline phosphatase; BCE, bone collagen equivalent.

[a] Serum BSAP was significantly ($P < 0.05$) different between the PTHrP 500 and 625 μg/d groups, but there were no differences between the PTHrP groups and placebo.

[b] Serum CTX was significantly different between the PTHrP 500 and 625 μg/d groups ($P < 0.05$), but there were no differences between the PTHrP groups and placebo.

occurred, additional subjects were studied at the previous lower dose, in groups of three, until a total of 10 subjects were accrued. PTHrP was self-administered sc in the anterior abdomen in volumes between 0.34 and 0.70 ml by the study subjects, and vials were returned for compliance assessment. Dietary calcium intake before enrollment was assessed by recall as shown in Table 1, and subjects were instructed not to exceed a total calcium intake of 1000 mg/d, including diet and calcium supplements, throughout the study.

Some subjects developed a local dermatological reaction after the injection (see *Results*). To determine whether this reaction might have been a result of the acetic acid used to aliquot the PTHrP (see next section), a second placebo group of five subjects was added for whom the placebo consisted of saline suspended in vials in which 1.0 ml of 10 mM acetic acid had been lyophilized. No significant differences in baseline demographics were observed between the original placebo group of 10 subjects and this second acetic acid placebo group of five, so the data from the two placebo groups were combined into a single placebo group of 15.

Preparation and administration of PTHrP

PTHrP (1-36) was synthesized using solid-phase synthesis, cleaved, purified, aliquotted, and assessed for purity and bioactivity as described in detail previously (14–23) as approved by the Food and Drug Administration (Investigation New Drug no. 49,175). Placebo vials contained either no PTHrP and no acetic acid (first 10 subjects) or contained 10 mM acetic acid that was then frozen and lyophilized exactly as the PTHrP-containing vials (five subjects).

Analyses

Serum and urine were analyzed for total calcium, ionized calcium, phosphorus, creatinine, intact PTH (1-84) (Advia Centaur; Seimens, Deerfield, IL), and 25 hydroxyvitamin D (IDS, Fountain Hills, AZ) in the Clinical Chemistry Laboratory at the University of Pittsburgh Medical Center. Fasting calcium excretion (FECa) was calculated as described (21). $1,25(OH)_2D$ was assayed using an RIA as described (24). Bone turnover markers were measured in single batches, and the intraassay coefficients of variation (CVs) are indicated below. Plasma osteocalcin was measured by RIA as described previously (25) (CV 4.5%). The amino-terminal telopeptides of procollagen 1 (P1NP) (CV 3.2%), the serum amino-terminal telopeptide of collagen-1 (NTX) (CV 5.4%), and carboxy-terminal telopeptide of collagen 1 (CTX) (CV 7.0%) were measured using commercial kits from Orion Diagnostics RIA (Espoo, Finland), Osteomark ELISA (Ostex International, Seattle, WA), and Crosslaps ELISA (Nordic Bioscience Diagnostics, Inc., Herlev, Denmark), respectively.

Sample size estimate

This study was a dose-finding study designed to examine the primary end points of dose-limiting toxicity of escalating doses of daily sc PTHrP. The primary outcomes were therefore safety measures with markers of bone turnover and calcium metabolism secondary outcomes. The group sample size of 10 at the maximal tolerated dose was selected based on safety data in our previous phase 1 studies (16, 17).

Statistics

A detailed description of the statistical methods are provided in the Supplemental Material, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Results

Study enrollment and demographics

All 15 placebo subjects and nine of 10 500 μg/d subjects completed the protocol (one subject receiving 500 μg/d

was discontinued on d 16 for protocol reasons, but her data through d 16 are included), as did the first three 625 μg/d subjects. The dose was then escalated, per protocol, to 750 μg/d. Of the initial six subjects receiving 750 μg/d, three developed mild hypercalcemia (see below). Study drug was terminated in these subjects. Per protocol, seven additional subjects were then studied at the next lower dose, 625 μg/d. Thus, there were 15 placebo subjects, 10 500 μg/d subjects, 10 625 μg/d subjects, and six 750 μg/d subjects. Compliance with study drug was 100% as assessed by count of used of vials. The average age, years after menopause, and baseline serum and urine chemistries, vitamin D metabolites, and markers of bone turnover for these subjects are shown in Table 1. As can be seen in the table, there were no significant baseline differences between the placebo subjects and the subjects treated with PTHrP. There were statistical differences between the 625 and 500 μg/d groups for bone-specific alkaline phosphatase and serum CTX.

Effects on markers of bone formation

All three PTHrP groups displayed statistically significant increases in osteocalcin (Fig. 1A). In contrast, the placebo group showed no changes. Interestingly, there was no apparent PTHrP dose effect on osteocalcin. Unexpectedly, at the 750 μg/d dose, osteocalcin increased initially ($P = 0.03$ on d 10 and 15) and then declined as the study progressed (P = ns at d 21). Bone-specific alkaline phosphatase did not change in any of the treatment groups (not shown), as reported previously (15, 16).

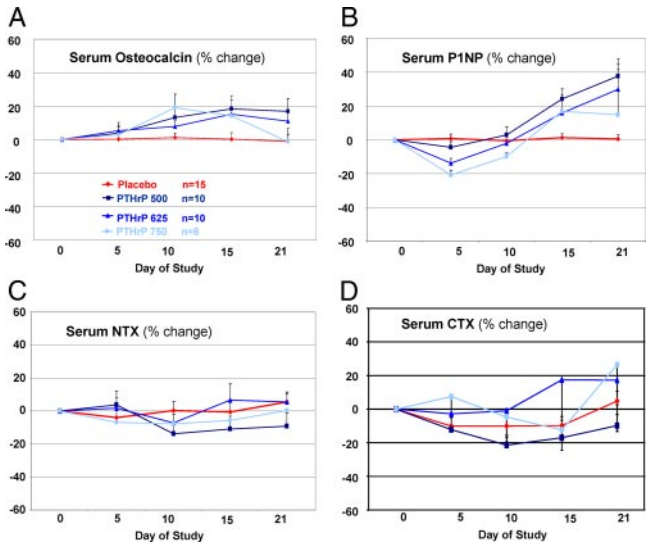

FIG. 1. Bone turnover markers In subjects treated with PTHrP for 3 wk: selective activation of bone formation. *Error bars* indicate SE of individual data points. In this and subsequent figures, when error bars are not visible, they are small and hidden within the corresponding symbol. The *color code* is shown in A. A, Serum osteocalcin measurements in the four groups. The changes at 500, 625, and 750 μg/d were statistically significant ($P < 0.05$ *vs.* placebo). B, Serum P1NP measurements. The changes at 500 and 625 μg/d were statistically significant ($P < 0.01$ *vs.* placebo and over time). C, Serum NTX measurements. There were no statistically significant differences over time. D, Serum CTX measurements. There were no statistically significant differences. Note that PTHrP treatment activated both of the two markers of bone formation but had no effects on resorption.

As observed with osteocalcin, P1NP measurements also remained stable in the placebo group. In contrast, P1NP increased in a statistically significant manner in the 500 and 625 μg/d PTHrP groups (Fig. 1B). The increment in the 750 μg/d did not achieve statistical significance. Interestingly, there was a statistically significant transient decline in P1NP at 625 and 750 μg/d at d 5.

Effects on markers of bone resorption

Bone resorption markers failed to increase in subjects at each of the three doses of PTHrP: serum NTX and CTX values in the 500, 625 and 750 μg/d groups were not significantly different from the placebo group (Fig. 1, C and D).

Effects on calcium, PTH, and vitamin D

Total and ionized serum calcium values on d 0–21 are shown in Fig. 2, A and B. There was no significant change in the mean serum calcium values in the placebo or 500 μg/d PTHrP groups. In the 500 μg/d group, none of the 10 subjects had a serum calcium above normal (>10.5 mg/dl). The highest serum calcium at any time in the study in this group was 10.4 mg/dl. The incidence of hypercalcemia was thus 0% in this group. In the 625 μg/d group, one of 10 subjects (11.1 mg/dl) exceeded the safety limit (>11.0 mg/dl) and was terminated on d 5. One additional subject developed a serum calcium of 10.7 mg/dl on d 5 but remained in the study without serum calcium going above the upper limit of normal at any later time points. The incidence of hypercalcemia in this group was thus either 10 or 20%, depending on the definition of hypercalcemia. The increase in mean serum calcium in the 625 μg/d group did achieve statistical significance ($P < 0.04$ *vs.* placebo d 5 and 10, $P < 0.005$ over time). The mean serum calcium also rose in the 750 μg/d group ($P < 0.004$ *vs.* placebo d 10), with three of the six subjects (50%) developing hypercalcemia (11.0, 11.1, and 11.2 mg/dl at d 5, 9, and 10) requiring that they discontinue the study before d 21. There was no significant change in ionized calcium in the 500 μg/d group, but both the 625 and 750 μg groups did develop statistically significant hypercalcemia ($P < 0.03$).

Serum total and ionized calcium values were studied in greater detail during an inpatient hospitalization on d 21 at the end of the study (Fig. 2, C and D). This included all of the placebo subjects, nine of 10 subjects receiving PTHrP at 500 μg/d, nine of 10 subjects at 625 μg/d, and three of six subjects at 750 μg/d. Only one subject in the 750 μg/d group had a serum calcium above the upper limit of normal on d 21. None of the subjects in the placebo, 500

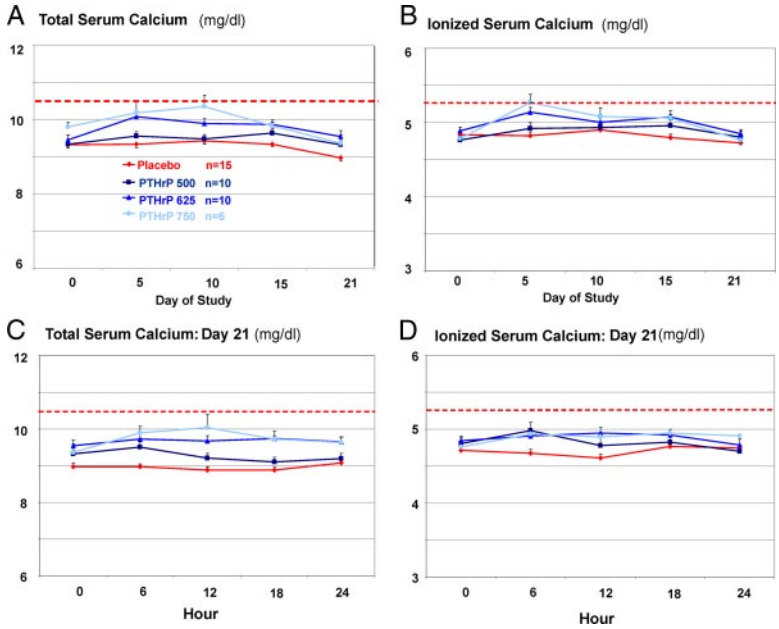

FIG. 2. Total and ionized serum calcium on d 1–21 and at multiple times throughout d 21. *Error bars* and *group symbols/colors* are as in Fig. 1. A, Total serum calcium over the 21 d of the study. PTHrP treatment had no statistically significant effect on total serum calcium in the 500 μg/d group but transient and mild effects on the 625 μg/d group ($P < 0.04$ *vs.* placebo d 5 and 10, $P < 0.005$ over time). Three of six subjects in the 750 group became mildly hypercalcemic ($P < 0.004$ *vs.* placebo on d 10) and were discontinued on d 5–10 according to protocol. Accordingly, values for the 750 μg/d shown on d 15–21 in this and subsequent figures do not include these three subjects. See text for details. B, Ionized serum calcium over the 21 d of the study. Again, there was no statistically significant change in the 500 μg/d group, but both the 625 and 750 μg groups did develop statistically significant hypercalcemia ($P < 0.03$). C, Total serum calcium over the final 24 h of the study. There was a statistically significant increase in the 750 μg/d group at 6 and 12 h after dose ($P < 0.05$ *vs.* placebo) but no statistically significant changes over time in the other groups. D, Ionized serum calcium over the final 24 h of the study. There were no statistically significant changes over time in any of the groups. Multiply total and ionized calcium values by 0.25 to convert milligrams per deciliter to millimoles per liter.

μg/d, or 625 μg/d had a serum calcium above normal (10.5 mg/dl) at any time on d 21. The mean total serum calcium rose in the 750 μg/d group at 6 and 12 h ($P < 0.05$ *vs.* placebo). Total mean serum calcium did not change during the course of the day in the subjects receiving placebo, 500 μg/d, or 625 μg/d. Similarly, there were no significant changes over time in any group for ionized calcium on d 21.

PTH (1-84) declined in all three PTHrP groups ($P < 0.01$ *vs.* baseline), and $1,25(OH)_2D$ unexpectedly increased over time in all three PTHrP groups ($P < 0.03$) (Fig. 3, A and B). Fasting FECa did not rise over time (Fig. 3C). In contrast, 24 h calcium excretion (Figs. 3D and 4) appeared to rise in a PTHrP dose-related manner from d 1 to d 21 but was significantly different from control only in the 625 μg/d group ($P < 0.0001$ *vs.* baseline, $P < 0.05$ *vs.* placebo). It is of note that only 10 of 15 placebo subjects and five of 10 subjects in the 500 μg/d had 24-h urine calcium measurements because these markers were added to the protocol after the initial 10 subjects had been enrolled. Subjects who were terminated before d 21 had a 24-h urine collected at the termination visit. Collectively, in the absence of evidence of bone resorption (Figs. 1, C and D, and 3C), these unexpected postabsorptive findings suggest that when hypercalcemia and postabsorptive hypercalciuria occur in response to PTHrP, they may result from intestinal calcium hyperabsorption, rather than bone resorption.

Serum phosphorus declined (nadir 3.2 ± 0.5 mg/dl at d 5 in the 750 μg/d group) but remained within the normal range in all subjects. There were no changes in serum creatinine.

Effects on cardiovascular homeostasis

Supplemental Fig. 1 displays the mean changes in systolic and diastolic blood pressure and pulse, supine and standing, during the last day of the study. No significant cardiovascular hemodynamic changes were observed. As shown in Table 2, one subject did develop postural hypotension and dizziness (systolic BP 73/45 mm Hg) when awakened and asked to stand at 0300 h, but vital signs were normal before and after.

Other AEs

A mild, local, erythematous, pruritic, and raised reaction developed at the site of injection in 12 subjects 3–5 d after injection (Table 2). The local reactions were mild, and no subject requested that the study be terminated. They resolved within 2–5 d but recurred in some cases after subsequent injections. No systemic allergic symptoms occurred. The reaction was not related to dose or batch of PTHrP. The pattern suggested a local, possibly chemical or toxic, reaction. To determine whether it was due to nonlyophilized acetic or triflouroacetic acid used in the synthesis and aliquotting of the PTHrP, the pH of the PTHrP vials after suspension in saline (as performed by the study subjects) and also the synthetic lots of PTHrP were measured. The pH was in the 3.2 range, in contrast to saline (5.5) and in contrast to the isoelectric point of the PTHrP (1-36) peptide (7.2). Furthermore, sterile vials that contained only lyophilized 10 mM acetic acid, but no PTHrP, had a pH after suspension with saline of 5.0. None of five additional placebo subjects (105 injections) developed a local reaction.

Discussion

PTHrP is a hormone produced in the mature osteoblast (8–13). PTHrP (but not PTH) is essential for attainment of

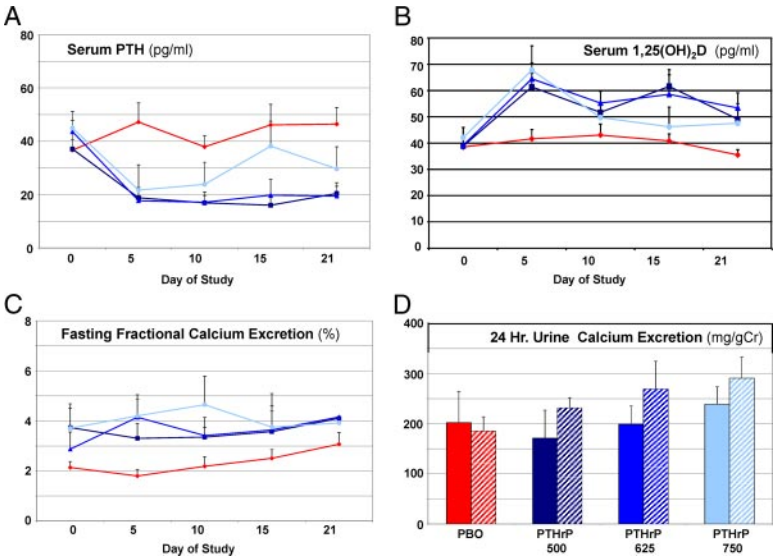

FIG. 3. Serum PTH, 1,25(OH)$_2$D, and urinary calcium excretion in the four groups. *Error bars* and *group symbols* are as in Fig. 1. A, Endogenous PTH (1-84) declined in all three PTHrP groups ($P < 0.01$ *vs.* placebo). To convert picograms per milliliter to picomoles, divide by 10. B, 1,25(OH)$_2$D increased in each PTHrP group during the study ($P < 0.03$ over time). To convert to picomoles per liter, multiply by 2.6. C, Fasting fractional calcium excretion. Whereas the four groups began the study with different values, reflecting the small numbers of subjects, there was no significant change over time in any of the four groups during the study. D, Twenty-four-hour calcium excretion on d 0 and d 21 of the study. Note that whereas fasting fractional calcium excretion did not increase with treatment (C), 24-h urine calcium excretion did tend to increase during the study, although these changes achieved statistical significance only in the 625 μg/d group ($P < 0.0001$ over time, $P < 0.05$ *vs.* placebo). Also note that only 10 of 15 placebo subjects and five of 10 subjects in the 500 μg/d and placebo groups had these measurements because they were added to the protocol after the initial 10 subjects were enrolled.

normal adult bone mass because haploinsufficiency or targeted disruption of the PTHrP gene within osteoblasts leads to osteoporosis (10–13). Intermittent administration of PTHrP to animal models of osteoporosis results in increments, exceeding normal, in BMD, trabecular bone volume, osteoblast number, and bone mineralization rates as well as biomechanical strength at the spine, femur, and tibia (20). Synthetic PTHrP can safely be administered to humans (14–19, 21–23) and achieves increments in BMD comparable with those elicited by PTH (19). In doses explored previously, this occurs without the development of either hypercalcemia or the other adverse effects associated with PTH (15–17). In contrast to PTH, therapeutically effective doses of PTHrP fail to activate bone resorption (15–17). This suggests that intermittently administered PTHrP, in contrast to PTH, may be a purely anabolic skeletal agent. The current study extends these prior studies. We report: 1) the maximum tolerable dose of PTHrP (1-36) in humans; 2) that even at the highest tolerable doses of PTHrP, bone turnover markers of osteoclast activity are not significantly increased; and 3) that whereas, as anticipated, hypercalcemia ultimately does occur with high doses, surprisingly, it may not result from bone resorption but may result instead from increases in 1,25(OH)$_2$D. Most importantly, this study provides essential dosing information for the design and performance of a direct head-to-head efficacy and safety study of PTHrP *vs.* approved doses of PTH.

We anticipated that PTHrP administration would increase markers of bone formation because we had previously shown that PTHrP is a skeletal anabolic agent in rats and humans: in rats using BMD and bone histomorphometry (20) and in humans in two studies, using bone turnover markers (15, 16). The current study confirms and extends these observations by using higher doses of PTHrP and the two current most widely used markers of bone formation, osteocalcin and P1NP. The absence of clear dose responsiveness for osteocalcin or P1NP may reflect the large intersubject variance in turnover markers (26, 27), the small numbers of subjects studied, and/or the limited magnitude of difference between the 500 μg/dose and the 625–750 μg doses. It is possible that the formation markers even declined with the highest (750 μg) dose of PTHrP on d 21 (Fig. 1). Unfortunately, toxicity at this dose prevented accrual of sufficient numbers of subjects to make firm interpretations.

Importantly, in this small study, PTHrP failed to activate bone resorption, as measured by significant changes in CTX and NTX, at any dose studied (Fig. 1). Because PTHrP was originally discovered as a result of its pathological (indirect) activation of osteoclastic bone resorption in humoral hypercalcemia of malignancy when secreted in a continuous manner (28, 29), we had assumed that as the PTHrP dose escalation progressed, we eventually would observe activation of bone resorption. In contrast to these expectations, PTHrP did not appear to activate bone resorption using the same biochemical markers. This contrasts with PTH, which routinely leads to activation of bone resorption markers (1–7). This unanticipated observation may relate to differences in how PTH and PTHrP interact with the common PTH-1 receptor (30) or may reflect the more rapid appearance and disappearance pharmacokinetics of PTHrP *vs.* PTH after sc injection (14). In the final analysis, however, it is possible that intermittently administered PTHrP may be a pure skeletal anabolic agent, in contrast to PTH, which is mixed skeletal anabolic and catabolic agent.

Whether intermittently administered PTHrP will activate bone resorption over the longer term remains unde-

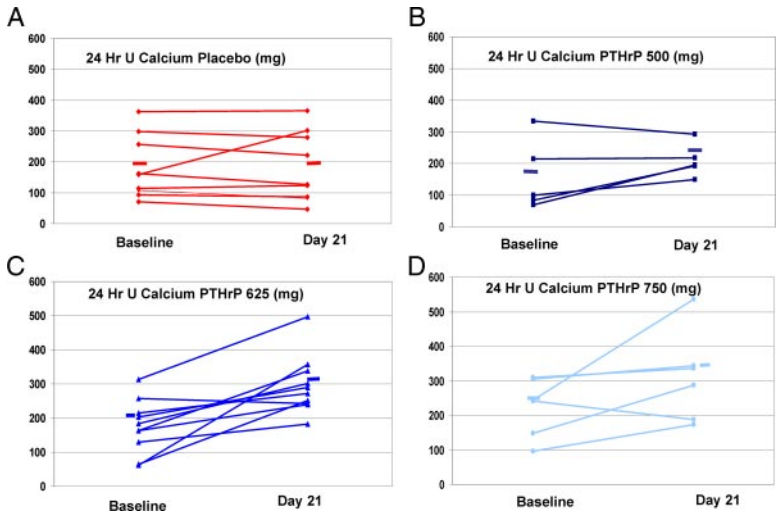

FIG. 4. Individual changes in 24-h urine calcium in the four groups. *Symbols* and *colors* are as in Fig. 1. Samples were collected at baseline on d 21 or termination of the study in subjects reaching DLT. *Horizontal bars* represent the mean for each time point. A, Individual placebo subjects (n = 10). B, Individual subjects receiving PTHrP 500 μg/d (n = 5). C, Individual subjects receiving PTHrP 625 μg/d (n = 10). D, Individual subjects receiving PTHrP 750 μg/d (n = 6).

fined. On the one hand, one can hypothesize that PTHrP does not, and will never, indirectly activate osteoclasts when administered intermittently in the doses reported and that if PTHrP were to activate osteoclasts, it would have been observed in the earlier 2-wk study (18), the current 3-wk study, and/or the previously reported 3-month study (16). Additionally, skeletal histomorphometry of rats treated with intermittent PTHrP for 6 months fails to reveal increases in osteoclast activity (20). In further support of this hypothesis, studies with PTH display increases in bone resorption as early as 2 (7) to 4 wk (1–6). On the other hand, it remains possible that PTHrP may activate bone resorption in human studies lasting more than 3 months. Having defined the complete therapeutic window of PTHrP, a direct head-to-head comparison of approved doses of PTH with therapeutically effective doses of PTHrP over longer periods of time can now be designed to address this important question.

TABLE 2. DLT and serious AEs in the study population

	Placebo (n = 15)	PTHrP 500 μg (n = 10)	PTHrP 625 μg (n = 10)	PTHrP 750 μg (n = 6)
Flushing	0	0	0	0
Nausea	0	0	0	0
Vomiting	0	0	0	0
Abdominal cramps	0	0	0	0
Dizziness	0	0	1[a]	0
Diaphoresis	0	0	0	0
Palpitations	0	0	0	0
Hypertension	0	0	0	0
Hypotension	0	0	1[a]	0
Bradycardia	0	0	0	0
Tachycardia	0	0	0	0
Hypercalcemia	0	0	1	3
Hypercalcuria	2	1	4	3
Dermal reaction	0	4	6	2
Other	0	0	0	0

[a] Both of these AEs occurred in the same subject when awoken and asked to stand at 0300 h.

Significant hypercalcemia (total serum Ca > 11.0 mg/dl) requiring discontinuation of the study drug was observed in three (50%) of the 750 μg/d subjects and one (10–20%) of the 625 μg/d subjects with one additional subject at 625 μg/d developing a single episode of mild hypercalcemia (serum Ca = 10.7 mg/dl on d 5). The incidence of hypercalcemia at 750 μg/d likely precludes this as a therapeutic dose in future studies (with one provision discussed below). In contrast, the 10–20% incidence at 625 μg/d is similar to that reported in the studies using the approved dose of PTH (1-34) (1-6), making this dose a candidate for future studies. Hypercalcemia did not occur in any of the 10 subjects at the 500 μg/d dose or in any of the eight subjects in the prior 3-month study at 400 μg/d (16). Thus, because the 400 μg/d dose has previously been shown to be effective in increasing BMD and because both the 400–500 μg/d doses are free of adverse effects and hypercalcemia, these are particularly attractive doses for future studies.

The increase in $1,25(OH)_2D$ is particularly unexpected because$1,25(OH)_2D$ is reduced in humoral hypercalcemia of malignancy (28) and because we recently documented that it also responds poorly in humans infused continuously with PTHrP (22, 23). This contrasts with patients with hyperparathyroidism (28) and subjects infused with PTH who display clear and rapid increments in $1,25(OH)_2D$ (22, 23). This discrepant responsiveness of PTH and PTHrP on the human kidney led Gardella and colleagues (30) to a recent reexamination of the binding kinetics of PTH and PTHrP to the human PTH-1 receptor. This demonstrated that although PTH and PTHrP associate with the receptor with similar kinetics, they dissociate with strikingly different kinetics, with PTHrP dissociating greater than 10 times more rapidly (30). We thus hypothesize that the large doses of PTHrP (500–750 μg/d) in the current study, compared with the much lower doses of PTH (20 μg/d) used for osteoporosis treatment, may override the rapid receptor dissociation and account for the increase in $1,25(OH)_2D$ observed herein. The increase in $1,25(OH)_2D$ also suggests that the direct anabolic effects of PTHrP on the skeleton may be indirectly supplemented via additional actions of $1,25(OH)_2D$ to augment osteoblastic bone formation as well as intestinal calcium

absorption (31, 32). Interestingly, in preliminary form, $1,25(OH)_2D$ concentrations also have been reported to increase in at least one PTH-osteoporosis treatment study (33). It is possible that 1,25 $(OH)_2$ D increases may mediate the hypercalcemia observed with higher doses of intermittent PTHrP.

One final observation regarding $1,25(OH)_2D$ may be important: $1,25(OH)_2D$ concentrations appeared to decline over time (Fig. 3B). This was also observed with PTH treatment of osteoporosis (33). These observations suggest that if hypercalcemia and hypercalciuria were to occur, they may attenuate over time, as $1,25(OH)_2D$ concentrations decline. Future studies should be designed to examine this possibility because even higher doses or PTHrP may be tolerated over longer time frames.

The most important adverse effect of PTHrP was a local erythematous reaction. For several reasons, this was likely a response to the low pH of the PTHrP formulation used. First, there were no systemic allergic symptoms (wheezing, anaphylactic symptoms or signs, rashes distant from the site of injection, arthralgias, *etc.*). Second, the appearance was typical of a local toxic reaction. Third, most of the local reactions took several days to develop, even after 2–3 wk of receiving the injections, instead of instantaneously after injection. Fourth, PTHrP is not a foreign material but a normal peptide hormone: a 50% incidence of reaction would be very high for an allergic phenomenon. Fifth, we have not observed such a reaction in multiple prior studies (14–19, 21–23). Sixth, we found that the peptide used in the current studies, when dissolved in saline or water, displayed a markedly acid pH, in contrast to placebo vials and saline vehicle and to the isoelectric point of PTHrP (1-36). Thus, we attribute the local reaction to incomplete removal of acid contaminants in the PTHrP preparation used. Future studies will need to use an optimized PTHrP synthetic formulation.

With the exception of the local dermal reaction, PTHrP was remarkably well tolerated. There were no important hemodynamic adverse effects, and none of the 26 subjects receiving PTHrP in the current study or 20 subjects receiving 500–1500 μg/d of PTHrP in prior studies (15–17) developed nausea, vomiting, flushing, or muscle cramping, adverse effects occasionally reported in association with PTH therapy (1).

Collectively, these and prior studies suggest that PTHrP may hold promise as a pure skeletal anabolic agent for the treatment of osteoporosis. Certain attributes of PTHrP (its possible pure anabolic effects with lack of bone resorption; its lack of nausea, muscle cramps, flushing, and hypercalcemia with doses up to 500 μg/d; and its lack of methionine residues that might be oxidized, thereby limiting stability of PTH) suggest that it may have advantages over PTH in the treatment of osteoporosis. These studies now permit effective design of a direct comparator study of PTH *vs.* PTHrP.

Acknowledgments

The authors thank the members of our Data Safety Monitoring Board (Drs. Elizabeth Shane, Robert M. Neer, B. Lawrence Riggs, Susan L. Greenspan, G. David Roodman, Steven Wisniewski, and Saul Mazolowski) for their continued support and analysis of these studies. We also thank Drs. James Elliot and Myron Crawford (Yale University) for synthesis and characterization of the peptide used in these studies. We also thank the staff of the Clinical Translational Research Center for helping to support these studies. We also thank the many study participants who volunteered their time and commitment to this study. Finally, we thank the members of Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research at the Federal Drug Administration for their support for these studies.

Address all correspondence and requests for reprints to: Mara J. Horwitz, M.D., Division of Endocrinology, Falk 560, University of Pittsburgh School of Medicine, 3601 Fifth Avenue, Pittsburgh Pennsylvania 15213. E-mail: horwitz@pitt.edu.

This work was supported by National Institutes of Health Grants DK 51081, DK 073039, and DK071158 as well as the University of Pittsburgh Clinical Translational Sciences Awards, National Institutes of Health/National Center for Research Resources/Clinical Translational Science Award UL1 RR024153 and MO-1 RR000056.

Disclosure Summary: M.B.T., A.G.-O., S.M.S., L.P., A.B., and C.M.G. have nothing to declare. M.J.H. was a previous consultant for Pfizer and is a consultant for Merck; B.W.H. is a consultant for Diasorin; and A.F.S. has ownership interest and is a board member for Osteotrophin L.L.C. and was a previous consultant for Pfizer.

References

1. **Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH** 2001 Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344:1434–1441
2. **Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero P, Bouxsein ML, Bilezikian JP, Rosen CJ** 2003 The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Engl J Med 349: 1207–1215
3. **Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM** 2003 The effects of parathyroid hormone, alendronate or both in men with osteoporosis. N Engl J Med 349:1216–1226
4. **Greenspan SL, Bone HG, Ettinger MP, Hanley DA, Lindsay R, Zanchetta JR, Blosch CM, Mathiesen AL, Morris SA, Marriott TB** 2007 Effect of recombinant human parathyroid hormone (1-84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis. Ann Int Med 146:326–339
5. **Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, Dempster D, Cosman F** 1997 Randomized clinical trial of the effect

of parathyroid hormone on vertebral bone mass and fracture incidence among postmenopausal women with osteoporosis. Lancet 350:550–555
6. **Cosman F, Nieves J, Zion M, Woelfert L, Luckey M, Lindsay R** 2005 Daily and cyclic parathyroid hormone in women receiving alendronate. N Engl J Med 353:566–575
7. **Hodsman AB, Fraher LJ, Watson PH, Ostbye T, Stitt LW, Adachi JD, Taves DH, Drost D** 1997 A randomized controlled clinical trial to compare the efficacy of cyclical parathyroid hormone versus cyclical parathyroid hormone and sequential calcitonin to improve bone mass in postmenopausal women with osteoporosis. J Clin Endocrinol Metab 82:620–628
8. **Philbrick WM, Wysolmerski JJ, Galbraith S, Holt E, Orloff JJ, Yang KH, Vasavada RC, Weir EC, Broadus AE, Stewart AF** 1996 Defining the physiologic roles of parathyroid hormone-related protein in normal physiology. Physiol Rev 76:127–173
9. **Strewler GJ** 2000 The physiology of parathyroid hormone-related protein. N Engl J Med 342:177–185
10. **Amizuka N, Karaplis AC, Henderson JE, Warshawsky H, Lipman ML, Matsuki Y, Ejiri S, Tanaka M, Izumi N, Ozawa H, Goltzman D** 1996 Haploinsufficiency of parathyroid hormone-related peptide results in abnormal postnatal bone development. Dev Biol 175:166–176
11. **Miao D, He B, Jiang Y, Lobayashi T, Soroceanu MA, Zhao J, Su H, Tong XK, Amizuka N, Gupta A, Genant HK, Kronenberg HM, Goltzman D, Karaplis AC** 2005 Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH (1-34). J Clin Invest 115:2401–2411
12. **Miao D, Li J, Xue Y, Su H, Karaplis AC, Goltzman D** 2004 Parathyroid hormone-related peptide is required for increased trabecular bone volume in parathyroid hormone-null mice. Endocrinology 145:3554–3562
13. **Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VLJ, Kronenberg HM, Mulligan RC** 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev 8:277–289
14. **Henry JG, Mitnick M, Dann PR, Stewart AF** 1997 Parathyroid hormone-related protein(1-36) is biologically active when administered subcutaneously to humans. J Clin Endocrinol Metab 82:900–906
15. **Plotkin H, Gundberg C, Mitnick M, Stewart AF** 1998 Dissociation of bone formation from resorption during two-week treatment with hPTHrP(1-36) in humans: potential as an anabolic therapy for osteoporosis. J Clin Endocrinol Metab 83:2786–2791
16. **Horwitz MJ, Tedesco MB, Gundberg C, Garcia-Ocaña A, Stewart AF** 2003 Short-term, high-dose PTHrP as a skeletal anabolic agent for the treatment of postmenopausal osteoporosis. J Clin Endocrinol Metab 88:569–575
17. **Horwitz MJ, Tedesco MB, Sereika SM, Garcia-Ocaña A, Bisello A, Hollis BW, Gundberg CM, Stewart AF** 2006 Safety and tolerability of subcutaneous PTHrP(1-36) in healthy human volunteers: a dose escalation study. Osteoporos Int 17:225–230
18. **Stewart AF, Mangin M, Wu T, Goumas D, Insogna KL, Burtis WJ, Broadus AE** 1988 A synthetic human parathyroid hormone-like protein stimulates bone resorption and causes hypercalcemia in rats. J Clin Invest 81:596–600
19. **Everhart-Caye M, Inzucchi SE, Guinness-Henry J, Mitnick MA, Stewart AF** 1996 Parathyroid hormone-related protein(1-36) is equipotent with parathyroid hormone(1-34) in humans. J Clin Endocrinol Metab 81:199–208
20. **Stewart AF, Cain RL, Burr DB, Jacob D, Turner CH, Hock JM** 2000 Six month daily administration of PTH and PTHrP peptides to adult ovariectomized rats markedly enhances bone mass and biomechanics: a comparison of human PTH(1-34), PTHrP(1-36) and SDZ-PTH-893. J Bone Miner Res 15:1517–1525
21. **Syed MA, Horwitz MJ, Tedesco MB, Garcia-Ocaña A, Wisniewski SR, Stewart AF** 2001 Parathyroid hormone-related protein (1-36) stimulates renal tubular calcium reabsorption in normal human volunteers: implications for the pathogenesis of humoral hypercalcemia of malignancy. J Clin Endocrinol Metab 86:1525–1531
22. **Horwitz MJ, Tedesco MB, Sereika SM, Hollis BW, Garcia-Ocaña A, Stewart AF** 2003 Direct comparison of sustained infusion of hPTHrP(1-36) versus hPTH(1-34) on serum calcium, plasma $1,25(OH)_2$ vitamin D concentrations and fractional calcium excretion in healthy human volunteers. J Clin Endocrinol Metab 88: 1603–1609
23. **Horwitz MJ, Tedesco MB, Sereika SM, Syed MA, Garcia-Ocaña A, Bisello A, Hollis BW, Rosen CJ, Wysolmerski JJ, Dann P, Gundberg C, Stewart AF** 2005 Continuous infusion of parathyroid hormone versus parathyroid hormone-related protein in humans: discordant effects on $1,25(OH)_2$ vitamin D and prolonged suppression of bone formation. J Bone Miner Res 20:1792–1803
24. **Hollis BW, Kamerud JQ, Kurkowski A, Beaulieu J, Napoli JL** 1996 Quantification of circulating 1,25(OH)2D by RIA with an 125-I-labled tracer. Clin Chem 42:586–592
25. **Gundberg CM, Nieman SD, Abrams S, Rosen H** 1998 Vitamin K status and bone health: an analysis of methods for the determination of "undercarboxylated" osteocalcin. J Clin Endocrinol Metab 83: 3258–3266
26. **de Papp AE, Bone HG, Caufield MP, Kagan R, Buinewicz, Chen E, Rosenberg E, Reitz RE** 2007 A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 40: 1222–1230
27. **Glover SJ, Gall M, Schoenborn-Kellenberger O, Wagener M, Garnero P, Boonen S, Cauley JA, Black DM, Delmas PD, Eastell R** 2009 Establishing a reference interval for bone turnover markers in 637 healthy, young, premenopausal women from the United Kingdom, France, Belgium and the United States. J Bone Miner Res 24:389–397
28. **Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE** 1980 Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and non-humoral groups. N Engl J Med 303:1377–1383
29. **Stewart AF, Vignery A, Silverglate A, Ravin ND, LiVolsi V, Broadus AE, Baron R** 1982 Quantitative bone histomorphometry in humoral hypercalcemia of malignancy: uncoupling of bone cell activity. J Clin Endocrinol Metab 55:219–227
30. **Dean T, Vilardaga JP, Potts Jr JT, Gardella TJ** 2008 Altered selectivity of parathyroid hormone (PTH) and PTH-related protein (PTHrP) for distinct conformations of the PTH/PTHrP receptor. Mol Endocrinol 22:156–166
31. **Sellmeyer DE, Black DM, Palermo L, Greenspan S, Ensrud K, Bilezikian J, Rosen CJ** 2007 Hetereogeneity in skeletal response to full-length parathyroid hormone in the treatment of osteoporosis. Osteoporos Int 18:973–979
32. **Samadfam R, Xia Q, Miao D, Hendy GN, Goltzman D** 2008 Exogenous PTH and endogenous 1,25-dihydroxyvitamin D are complimentary in inducing an anabolic effect on bone. J Bone Miner Res 23:1257–1265
33. **Cosman F, Dawson-Hughes B, Chen P, Krege JH** 2007 Changes in 25-hydroxy- and 1,25-dihydroxy-vitamin D during treatment with teriparatide. J Bone Miner Res 22(Suppl 1):S210