Short-term Effects of Synthetic Human Parathyroid Hormone-(1–34) Administration on Bone Mineral Metabolism in Osteoporotic Patients

DAVID M. SLOVIK, ROBERT M. NEER, and JOHN T. POTTS, JR., Endocrine Unit, Massachusetts General Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT Since studies in animals and humans have shown that parathyroid hormone can stimulate bone formation and increase trabecular bone, and patients with primary and secondary hyperparathyroidism may exhibit osteosclerosis, we evaluated the effect of short-term administration of human parathyroid hormone, hPTH-(1–34), in patients with osteoporosis.

Six patients with osteoporosis underwent detailed studies including blood and urinary measurements of calcium, phosphate, and magnesium; 47Ca kinetic studies; and 18-d balance studies before and during the short-term administration (3–4 wk) of a daily subcutaneous injection of hPTH fragment 1–34 given as 450 or 750 U/dose.

The mean fasting plasma calcium values rose slightly after hPTH-(1–34) administration, primarily in the high-dose group. There was no difference in the mean fasting plasma inorganic phosphate levels. The mean daily urinary excretion of calcium and phosphate was significantly increased in patients given the higher dose.

In patients given 450 U, net intestinal calcium absorption increased, phosphate absorption increased, calcium balance improved, and phosphate balance improved. In patients given 750 U, calcium balance and phosphate balance worsened.

47Ca kinetic studies showed a minimal increase in bone accretion rate, a decrease in the mean transit time of calcium in the exchangeable pools, and a decrease in the exchangeable-pool size. In all six patients there was an increased renal clearance of 47Ca as a result of hPTH-(1–34) administration.

These studies indicate that low doses of parathyroid hormone may promote bone formation, whereas higher doses clearly have an adverse effect on the skeleton.

INTRODUCTION

Parathyroid hormone has multiple direct and indirect effects upon the skeleton, including increased synthesis and destruction of bone matrix, and increased uptake and release of bone mineral (1, 2). It is well known that high doses of parathyroid hormone are catabolic to bone in animals, and excessive secretion of endogenous hormone reduces bone mass in humans and animals. The dose dependence of parathyroid hormone’s effects upon bone metabolism in the human skeleton has not been adequately investigated, but multiple investigators have shown that low doses of parathyroid hormone are anabolic for trabecular bone in young rats (3–7). Repeated injections of the hormone increase trabecular bone in such animals whether they are intact or previously thyroparathyroidectomized (6). It has been impossible to evaluate the effects of low doses of parathyroid hormone on trabecular bone mass in humans in the past because the only available material was bovine parathyroid hormone, which provokes neutralizing antibodies in humans after several weeks of injections (8). It is known, however, that osteosclerosis occasionally develops in patients with mild primary hyperparathyroidism (9, 10) and frequently occurs in patients with secondary hyperparathyroidism due to uremia (11–13).

In recent years the sequence of human parathyroid hormone has been determined (14–16). The biologically active amino-terminal portion of the human parathyroid hormone, hPTH-(1–34), as well as various biologically active analogs and fragments of para-

Address correspondence to Dr. Slovik.

Received for publication 24 October 1979 and in revised form 17 July 1981.

1Abbreviations used in this paper: hPTH, human parathyroid hormone.
thyroid hormone have been synthesized, and are being used to characterize details of the hormones actions on renal and skeletal tissues (17–19). Definitive clinical studies have benefited from the availability of the synthetic peptide hPTH-(1–34), which is a pure compound, in contrast to the previously available bovine hormone preparation, which is a highly impure parathyroid extract with variable biological potency because of the presence of multiple hormone fragments of various strengths and biological activity. Synthetic hPTH-(1–34) produces all the known biological effects of the intact 84-amino acid hormone in vivo (19), and increases bone mass when added in vitro to tissue cultures of embryonic mouse radiae (20). A multicenter study had been organized to evaluate the effects of this peptide on bone mass in elderly humans with idiopathic osteoporosis. These studies show that the hPTH-(1–34) increases bone turnover in such patients and also increases trabecular bone in paired iliac-crescent biopsies (21–24).

Optimum use of synthetic hPTH-(1–34) to stimulate bone turnover and increase bone mass in humans requires more detailed knowledge of the dose dependence of the effects of this peptide on bone in humans. The effects of different dosages of this synthetic peptide on bone mineral metabolism in humans have been reported only by Reeve et al. (25), who studied the effects of three different doses of hPTH-(1–34) on calcium balance, renal tubular function, and skeletal turnover. Only two patients were studied, however, and the peptide dose was increased every 8 d. They concluded that the highest dose tended to cause bone breakdown. We report here more extended studies on six patients, each given either 450 or 750 U of hPTH-(1–34) daily for 18 d.

METHODS

Subjects. Six patients (five females and one male), 50–70 yr of age with osteoporosis were studied. The diagnosis was established by iliac-crescent bone biopsy (26), metacarpal measurements (27), femoral trabecular index (Singh score) (28), gamma photon absorptiometry using the Norland-Cameron bone mineral analyzer (29, 30), and history of vertebral crush fractures (Table I).

Bone biopsies in all patients except patients 2 and 5 were done after double-tetracycline labeling and analyzed by detailed histomorphometric analyses (26), which are listed in Table II. Patient 2 did not present with vertebral crush fractures; however, the metacarpal cortical area and bone densitometry (distal radius) were >1 SD below the normal mean, the femoral trabecular index was reduced to 3.0, and a bone biopsy revealed osteoporosis. Plasma immunoreactive parathyroid hormone levels were determined using GP1 antiserum, and results are expressed as microliter equivalents of a standard hyperparathyroid human plasma (31); values were normal in all patients. Serum 25-hydroxylated vitamin D, measured by a competitive protein-binding technique (32), was well within the normal range in all patients (44±16; mean±SD).

All patients had normal levels of serum alkaline phosphatase and had not sustained any fractures for at least 3 mo before they entered the study. All patients had a complete medical evaluation before entering the study. All had normal urinalysis, blood urea nitrogen, and serum creatinine. Creatinine clearance was normal in all patients when corrected for age (33), except in patient 2 who had a slightly lower, but stable, value and no other evidence of renal abnormality. There was no history of any prior gastrointestinal surgery. In each patient the serum carotenoids, d-xylene tests, and 72-h stool-fat values were normal. All liver-function tests were normal. Adrenal and thyroid function were normal.

None of the patients had what has been termed "high-turnover osteoporosis" (26, 34, 35) since immunoreactive parathyroid hormone levels, urinary hydroxyproline excretion, and alkaline phosphatase values were normal, and osteoblastic apposition rates were low or low–normal in the two patients who had an increased percentage of resorptive surface on trabecular bone biopsies.

Patient 1 had been started on a regimen of conjugated estrogens 10 mo earlier; the dosage was 1.25 mg/d for 21 d, followed by a 7-d period without this drug every month. Patient 2 had been taking 2,000 U of vitamin D3 and 200 mg 4 times/d of calcium citrate for the preceding 2 yr and was also receiving (i.m.) testosterone enanthate (90 mg) and estradiol valerate (1 mg) monthly for the 2 yr before entering the study. No other patient was on any vitamin D supplement except in the form of a multivitamin (400 IU/d), and no patient received any other drug known to affect calcium or phosphate homeostasis.

Experimental design. All studies were performed with the informed consent of the individual. Each patient was admitted to the Clinical Research Center of the Massachusetts General Hospital. All patients were ambulatory. Their daily routine consisted of light physical activity without prolonged bedrest. Each subject's diet was of constant composition and low hydroxyproline content. A constant amount of distilled water was given daily. All urine and feces as well as any vomitus were saved. The diets were chosen according to the patient's preferences and approximated their customary intake of calcium and other nutrients.

The experimental design used each patient as their own control. Since those patients on estrogens, androgens, or vitamin-D therapy had been on these medications for many months or years before being studied, it is unlikely that changes seen between the control and hormone administration periods were due to these concomitant medications.

A control study consisted of an initial run-in period of 5–7 d to allow a steady state to be obtained. An 18-d 4Ca kinetic study was then performed, along with an 18-d study, divided into three 6-d periods of calcium, phosphate, and magnesium balance. At the end of this control period, a synthetic fragment of hPTH-(1–34) was given daily by subcutaneous injection. After hPTH-(1–34) was administered for 7–10 d, a second 18-d kinetic study was performed, during which hPTH-(1–34) was continued as a daily injection at 0600 h. All food and medication were withheld on the day that each 4Ca kinetic study began until 6 h after the 4Ca injection. Thereafter, the patient's total 24-h intake of food and medication was administered during the remaining 14–16 h. Treatment balance studies, begun on the day hPTH-(1–34) was started, consisted of four 6-d periods.

The balance technique followed was essentially that described by Reifenstein et al. (36). All fecal specimens were initially frozen, and when ready for homogenization, were pooled into 6-d collection periods in a paint can, to which was added 95% alcohol, distilled water, and cellulose gum (Hercules Inc., Wilmington, Delaware). Lead balls (2–3 cm)
Table I
Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Height</th>
<th>Weight</th>
<th>Fracture history</th>
<th>Bone densitometry</th>
<th>Metacarpal cortical area</th>
<th>Singh score</th>
<th>25-OHD (ng/ml)</th>
<th>Immuno-reactive PTH (μeq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>F</td>
<td>146</td>
<td>49.5</td>
<td>T8 L1, 3–5</td>
<td>0.55</td>
<td>0.40</td>
<td>72</td>
<td>3.5</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>F</td>
<td>154</td>
<td>37.8</td>
<td>Ankle Kyphosis</td>
<td>0.66</td>
<td>0.33</td>
<td>76</td>
<td>3.0</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>F</td>
<td>151</td>
<td>36.6</td>
<td>T5–8 T11–12 L1</td>
<td>0.48</td>
<td>0.25</td>
<td>64</td>
<td>2.0</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>F</td>
<td>142</td>
<td>35.2</td>
<td>T6–12 L1–5</td>
<td>0.44</td>
<td>0.22</td>
<td>71</td>
<td>2.0</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>M</td>
<td>171</td>
<td>65.8</td>
<td>T5–12 L1–5 hip</td>
<td>0.79</td>
<td>0.41</td>
<td>59</td>
<td>3.0</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>F</td>
<td>138</td>
<td>50.9</td>
<td>T5–12 L1–2</td>
<td>0.49</td>
<td>0.28</td>
<td>—</td>
<td>—</td>
<td>46</td>
</tr>
</tbody>
</table>

* Mean±SD.

were then added and the entire mixture was shaken on a paint mixer until homogeneous. Weighed aliquots of the fecal homogenate were taken and then underwent perchloric-nitric acid digestion and subsequent analysis of calcium, phosphate, and magnesium. 

Table II
Histomorphometric Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Trabecular osteoid volume</th>
<th>Trabecular osteoid surfaces</th>
<th>Thickness index osteoid seams</th>
<th>Trabecular resorption surface</th>
<th>Osteoclast No./mm²</th>
<th>Trabecular calcification rate</th>
<th>Cortical bone calcification rate</th>
<th>Bone biopsy diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0 (1.6±0.7)</td>
<td>15.3 (8.6±3.9)</td>
<td>19.7 (18.5±2.5)</td>
<td>n.m.</td>
<td>n.m.</td>
<td>(0.72±0.12)</td>
<td>(0.80±0.26)</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>3</td>
<td>3.5 (1.6±0.7)</td>
<td>28.7 (8.6±3.9)</td>
<td>12 (18.5±2.5)</td>
<td>5.6 (3.6±1.1)</td>
<td>(&lt;0.20)</td>
<td>(0.72±0.12)</td>
<td>(0.80±0.26)</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>4</td>
<td>1.2 (2.2±1.7)</td>
<td>14.7 (12.1±7.5)</td>
<td>8.2 (17.7±4.0)</td>
<td>3.3 (3.6±1.1)</td>
<td>(&lt;0.20)</td>
<td>N.L.</td>
<td>N.L.</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>6</td>
<td>2.3 (1.6±0.7)</td>
<td>23.3 (8.6±3.9)</td>
<td>9.8 (18.5±2.5)</td>
<td>7.4 (3.6±1.1)</td>
<td>(&lt;0.20)</td>
<td>0.62 (0.72±0.12)</td>
<td>0.65 (0.80±0.26)</td>
<td>Osteoporosis</td>
</tr>
</tbody>
</table>

N.L., No tetracycline label seen, though medication taken; n.m., nonmeasurable. Normal values are in parentheses. (mean±SD).

Short-term Effects of Human Parathyroid Hormone-(1–34) in Osteoporosis 1263
μCi of 47CaCl2 (37) with 50 mg chromium sesquioxide carrier was given daily by mouth in a single capsule to four patients beginning several days before admission and throughout the hospitalization. In patients 2 and 4, stable chromium alone was used as a fecal recovery marker. Duplicate diet aliquots were analyzed twice during each 6-d period for calcium, phosphate, and magnesium; after first being dried at 97°C, the aliquots were ashed in a muffle furnace at 480°C, dissolved in concentrated HCl, ashed again at 480°C, and redissolved in 1 N HCl. The urine collected each 24-h was pooled into bottles containing 10 ml of 6 N HCl from 0600 through 0600 h, and each collection was analyzed for calcium, phosphate, magnesium, creatinine, and hydroxyproline.

Three times weekly during the study periods, fasting plasma was obtained for calcium, phosphate, magnesium, and creatinine determinations. In addition, on these same days, renal clearance of phosphate and creatinine was measured from 0600 through 0900 with the patient supine and fasting, to calculate the maximum tubular reabsorption capacity divided by the glomerular filtration rate (TmP/GFR) (38). Plasma and urine calcium and magnesium were analyzed by atomic absorption, and plasma and urine phosphate and creatinine by spectrophotometer (39, 40). Urine hydroxyproline was analyzed by the method of Kivirikko et al. (41).

47Ca kinetic studies were performed in each balance study with the patient in the fasting state (42). 47CaCl2 dissolved in 5–10 ml of sterile acidified isotonic saline was given by rapid injection into an antecubital vein. Plasma samples were taken from the opposite antecubital vein through an indwelling catheter at 2, 4, 6, 8, 11, 15, 20, 25, 30, 35, 45, 60, 80, 100, 120, 180, 240, 300, 360, 480, 600, and 720 min, and at 1, 1.5, 2, 3, and 4 d after the isotopic injection. These samples were then analyzed for radioactivity and stable calcium. The radioactivity in the plasma was measured with a 3-in sodium iodide well crystal. The radioactivity was expressed as the fraction of the injected dose per gram of calcium. Urine was placed into 24-h pooled collections as described above and counted in 2,000-ml Marinelli-type vessels using a 3-in sodium iodide solid crystal. Urine specific activity was expressed as the fraction of the injected dose per gram of calcium. The cumulative fraction of the injected dose was also calculated. Fecal specimens were collected for 18 d after the isotope injection and pooled into three 6-d pools which were individually counted in a fashion similar to that used for urine. The radioactivity recovered was expressed as the cumulative fraction of the injected dose. 47Ca counting was done above 0.4 million electron volts (MEV) to eliminate radiation from 47Sc, the radioactive decay product of 47Ca. We counted 45Cr and 47Ca radioactivity in the feces simultaneously, using separate 45Ca and 47Cr standards and a 1028-channel gamma spectrometer, Norland/Ino-Tech, Fort Atkinson, Wis. The 47Ca was counted with a window of 0.278–0.356 MEV and 45Ca using a window of 0.460–1.440 MEV, and all count rates were corrected for isotope crossover. All samples were counted for a length of time to allow a 2–3% coefficient of variation for the net count rate (43). Endogenous fecal calcium is estimated from the amount of radioactivity appearing in the feces after the intravenous injection of 47Ca. It was calculated as follows:

endogenous fecal calcium (mg/d) = (fraction of injected dose in feces) / (mean daily urine calcium (mg/d)) - (fraction of injected dose in urine)

The net intestinal absorption represents the difference between dietary intake and fecal content of calcium or phosphate. The true calcium absorbed represents the amount of dietary calcium actually absorbed, correcting for endogenous fecal calcium. It is calculated as follows (all terms figured in milligrams per day):

True calcium absorption = dietary intake + endogenous fecal calcium – measured fecal calcium.

Bone resorption was calculated as the difference between bone accretion and calcium balance.

The hPTH-(1–34) used in this study was synthesized according to the Niall sequence; purified to homogeneity, and packaged in sterile single-dose ampoules. Five of the patients were treated with a single batch of synthetic hPTH-(1–34). The sixth patient (patient 2) was treated with a different batch of synthetic hPTH-(1–34) made earlier. The potency of this hormone relative to that used for the other patients was assured by assay of each ampoule of hormone against appropriate standards in the chick hypercalcemic assay (44), with preparation 75-596 used as a standard. The potency of both batches of hormone remained stable.

Data analysis. Blood and urine samples were compared in the control and treatment period in individual patients by using an unpaired t test with allowance for possible unequal variances. When comparing the effects of hPTH-(1–34) on calcium, phosphate, and magnesium balance, and in intestinal absorption, statistical significance was assessed by a nested analysis of variance with two levels for treatment (control vs. peptide) and dose (high vs. low) and seven measures for each subject. The effects of treatment were tested separately at each dose, and the dependence of the treatment effect on the peptide dose was also tested, the variance within cells being used as the error term (45). In analyzing the net intestinal absorption and balance studies, the 6-d periods were used for each patient rather than the mean of the studies, so that the analysis of variance would take into account experimental variations in balance and absorption. 47Ca kinetic studies were analyzed according to a noncompartmental model (46). The turnover time of calcium in the exchangeable pools of bone was determined by the method described by Reeve (47).

RESULTS

No patient experienced symptoms from the hormone administration, nor were any toxic renal, hepatic, hematologic, or other effects detected.

Calcium metabolism

Blood. The mean levels of fasting plasma calcium were slightly but significantly higher (P < 0.01) after hPTH-(1–34) administration (9.42 ± 0.04 mg/dl) than those observed in the control period (9.29 ± 0.04 mg/dl) (Table III). This rise appeared to be primarily attributable to those patients receiving the higher dose of hPTH-(1–34). All levels of plasma calcium remained in the normal range except patient 5, in whom they rose to a maximum of 10.7 mg/dl 6 h after injection of hPTH-(1–34) but returned to normal within 12 h after hPTH-(1–34) injection.

Urine. An increase in urinary calcium excretion was evident 3–4 d after beginning hPTH-(1–34) therapy (Table III and Fig. 1). In those patients receiving the low dose of hPTH-(1–34), calcium excretion increased initially but after 2 wk returned towards the control values and was then not statistically significant.
from base line. On the other hand, patients receiving the high dose of hPTH-(1-34) had a sustained increase in calcium excretion which leveled off after 2 wk (Fig. 1). The mean increase over control urine calcium excretion was 92 mg/d (P < 0.01). After the plateau in calcium excretion, the mean urine calcium excretion in these patients was 315±17 mg/d vs. control values of 214±7 mg/d (P < 0.001 vs. control and P < 0.001 vs. low-dose group at this time). Hypercalcuiuria developed in patient 5 during administration of the higher dose of hPTH-(1-34).

**Intestinal absorption.** Net intestinal calcium absorption (intake minus feces) showed an overall mean increase of 204% in the low-dose group (P < 0.01) (Table III and Fig. 2). Each patient in this group had an increase in calcium absorption efficiency, net intestinal absorption, and true intestinal absorption (corrected for endogenous fecal calcium) as a result of hPTH-(1-34) administration. In contrast, patients receiving the high dose tended to show a worsening in intestinal calcium absorption as a result of hPTH-(1-34), but this was not statistically significant.

**Balance.** There were significant changes in calcium balance in both the patients receiving the low dose and those receiving the high dose (Table III and Fig. 2). The changes were in opposite directions, however, patients receiving the low dose showed a mean improvement in calcium balance of 110%. Comparison using the individual periods during the control study and when 450 U hPTH-(1-34) was administered, each patient acting as his/her own control, showed that the mean increase of 85 mg/d was statistically significant (P < 0.05) by analysis of variance.

On the other hand, the high dose of hPTH-(1-34) resulted in a negative calcium balance. The decrease in calcium balance averaged 149 mg/d.

In no patient did endogenous fecal calcium change more than 20 mg/d as a result of hPTH-(1-34) administration, and urine calcium stayed the same or increased slightly during hPTH-(1-34) administration. Thus, the improvement in calcium balance seen on the lower dose is explained by an increase in net intestinal absorption (Fig. 2).

**47Ca turnover.** As determined by 47 calcium kinetic studies, there was no substantial change in skeletal accretion (Table IV). The biggest change was seen in patient 5 who had an increase of 59% (127 mg/d) in the accretion rate. In all other patients accretion changed by <42 mg/d. In all patients, there was a decrease in the mean transit time of calcium in the exchangeable pools. In addition, in five of the six patients there was a decrease in the size of the exchangeable calcium pool. Bone resorption decreased by a mean of 30% in the three patients receiving low doses of

---

**TABLE III**

**Effects of Short-term hPTH-(1–34) Administration on Calcium Metabolism**

<table>
<thead>
<tr>
<th>Patient</th>
<th>hPTH-(1-34)</th>
<th>Plasma calcium</th>
<th>Urine calcium</th>
<th>Intake</th>
<th>True absorption*</th>
<th>Net intestinal absorption (intake − feces)</th>
<th>Absorption efficiency</th>
<th>Balance</th>
<th>Change in balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/d</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>%</td>
<td>mg/d</td>
<td>mg/d</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>9.37±.05</td>
<td>213±4</td>
<td>933</td>
<td>139</td>
<td>52</td>
<td>15</td>
<td>−161</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td>9.36±.04</td>
<td>267±7§</td>
<td>920</td>
<td>375</td>
<td>295</td>
<td>41</td>
<td>+28</td>
<td>+189</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>8.93±.10</td>
<td>159±4</td>
<td>872</td>
<td>135</td>
<td>132</td>
<td>16</td>
<td>−27</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td>9.15±.10§</td>
<td>178±8</td>
<td>873</td>
<td>233</td>
<td>206</td>
<td>27</td>
<td>+28</td>
<td>+55</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>9.48±.06</td>
<td>254±2</td>
<td>1414</td>
<td>173</td>
<td>45</td>
<td>12</td>
<td>−209</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td>9.70±.10</td>
<td>274±5§</td>
<td>1422</td>
<td>208</td>
<td>85</td>
<td>15</td>
<td>−189</td>
<td>+20</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>9.11±.05</td>
<td>168±3</td>
<td>1440</td>
<td>128</td>
<td>3</td>
<td>9</td>
<td>−165</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td></td>
<td>9.34±.07</td>
<td>237±6§</td>
<td>1446</td>
<td>−26</td>
<td>−156</td>
<td>−96</td>
<td>−393</td>
<td>−228</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>9.13±.03</td>
<td>275±5</td>
<td>960</td>
<td>313</td>
<td>50</td>
<td>33</td>
<td>−225</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td></td>
<td>9.47±.07§</td>
<td>410±14§</td>
<td>980</td>
<td>269</td>
<td>32</td>
<td>27</td>
<td>−378</td>
<td>−153</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>9.55±.05</td>
<td>200±4</td>
<td>1073</td>
<td>184</td>
<td>66</td>
<td>17</td>
<td>−134</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td></td>
<td>9.61±.06</td>
<td>232±4§</td>
<td>1075</td>
<td>144</td>
<td>33</td>
<td>13</td>
<td>−199</td>
<td>−65</td>
</tr>
</tbody>
</table>

All values mean±SEM.
Normal plasma calcium is 8.5–10.5 mg/dl.
* Intake minus feces, corrected for endogenous fecal calcium.
† (True absorption/intake) × 100.
§ P < 0.01 (t test, control vs. Rx).
* P < 0.05 (t test, control vs. Rx).
hPTH-(1–34) and increased by a mean of 52% in the three patients receiving the higher dose. Because of the small number of determinations, no statistical tests were feasible. As shown in Fig. 3, there was a greater cumulative excretion of the injected \(^{45}\)Ca in all patients as a result of hPTH-(1–34) treatment, amounting to an increase of 5.2% in the patients receiving the low dose and a similar increase (7.2%) in those receiving the high dose. These changes were entirely due to increased renal \(^{45}\)Ca excretion, which occurred despite a lower plasma \(^{45}\)Ca/total concentration in five of the six patients (Fig. 3).

Serum alkaline phosphatase and plasma creatinine levels did not change with hPTH-(1–34) administration and remained normal.

Hydroxyproline excretion increased significantly \((P < 0.01)\) only in patient 5, who was receiving the higher dose.

**Phosphate metabolism**

**Blood.** Although in the group as a whole, there was no statistically significant difference in the mean level of fasting plasma inorganic phosphate as a result of hPTH-(1–34) administration \((3.57 \pm 0.07\) control vs. \(3.60 \pm 0.10\) treatment), statistically significant increments were observed in patients 4 and 5; patient 6 had a significant decrease (Table V). All three of these patients were receiving the higher dose of hPTH-(1–34). In no patient was hypophosphatemia produced.

**Urine.** The mean daily urinary excretion of phosphate was significantly increased in the patients receiving the higher dose of hPTH-(1–34) \((P < 0.01); \text{mean increase } 87\text{ mg/d}\) (Table V and Fig. 1). Urinary phosphate excretion did not increase in patients receiving the lower dose of hormone. Patient 2 had a low 24-h urinary phosphate excretion level that did not change with hPTH-(1–34) administration. This patient's low urinary phosphate probably reflects her habitually low dietary intake of phosphate, since she had no osteomalacia on bone biopsy, and had normal circulatory levels of 25-(OH)D and parathyroid hormone.

In all patients there was a fall in the TmP/GFR as a result of hPTH-(1–34) administration. This was statistically significant at each dose level (low dose: \(P < 0.001\); \(3.4 \pm 0.2\) control vs. \(2.4 \pm 0.1\) treatment; high dose: \(P < 0.001\); \(3.8 \pm 0.1\) control vs. \(3.2 \pm 0.1\) treatment).

In all patients there was a significant \((P < 0.01)\) increase in the amount of phosphate excreted during the 3 h \((0600–0900)\) after hormone administration as compared with the total 24-h urinary phosphate excretion. The low-dose group showed a mean increase

**FIGURE 1** Mean daily urinary excretion of calcium, phosphate, and magnesium in patients given low and high doses of hPTH-(1–34). Immediately after an 18-d control period, subcutaneous injections of hPTH-(1–34) were administered each day at 0600 h as indicated by the bar at the top of the figure. ●, Low dose of hPTH-(1–34); ▲, the higher dose.

**FIGURE 2** Effects of daily hPTH-(1–34) administration on calcium, phosphate, and magnesium balance, and on net intestinal absorption (intake minus feces) in each individual patient. Each datum point represents the mean of the individual periods in the control and treatment study. ○, Subjects given low dose of hPTH-(1–34); △, subjects given the higher dose. Nos. 1–6 refer to the individual patients and correspond to the data in the text.
### TABLE IV
Effects of Short-term hPTH-(1–34) Administration on Bone Turnover

<table>
<thead>
<tr>
<th>Patient</th>
<th>hPTH-(1–34)</th>
<th>Skeletal calcium accretion</th>
<th>Skeletal calcium release</th>
<th>Mean transit time</th>
<th>Exchangeable pool</th>
<th>Serum alkaline phosphatase</th>
<th>Urine hydroxyproline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ud</td>
<td>mg/d</td>
<td>d</td>
<td>mg Ca</td>
<td>Bodansky U &lt;7</td>
<td>mg/d</td>
<td>mg/d</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>194</td>
<td>355</td>
<td>1.35</td>
<td>3,217</td>
<td>6.1±0.5</td>
<td>17±1</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>200</td>
<td>172</td>
<td>1.24</td>
<td>2,806</td>
<td>4.2±0.8</td>
<td>18±0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>210</td>
<td>237</td>
<td>1.26</td>
<td>3,432</td>
<td>3.5±0.0</td>
<td>11±0</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>168</td>
<td>140</td>
<td>1.13</td>
<td>2,600</td>
<td>3.9±0.0</td>
<td>8±0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>142</td>
<td>351</td>
<td>1.27</td>
<td>3,143</td>
<td>3.0±0.1</td>
<td>15±1</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>163</td>
<td>352</td>
<td>1.14</td>
<td>2,749</td>
<td>3.4±0.4</td>
<td>17±1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>89</td>
<td>254</td>
<td>1.10</td>
<td>2,520</td>
<td>3.3±0.0</td>
<td>20±1</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>80</td>
<td>473</td>
<td>1.09</td>
<td>2,662</td>
<td>5.7±0.0</td>
<td>14±1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>214</td>
<td>439</td>
<td>1.44</td>
<td>7,500</td>
<td>3.3±0.2</td>
<td>30±1</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>341</td>
<td>719</td>
<td>1.10</td>
<td>5,196</td>
<td>3.0±0.3</td>
<td>45±2</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>279</td>
<td>413</td>
<td>1.25</td>
<td>3,241</td>
<td>5.5±0.3</td>
<td>22±6</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>297</td>
<td>496</td>
<td>1.23</td>
<td>3,223</td>
<td>5.5±0.0</td>
<td>20±0</td>
</tr>
</tbody>
</table>

All values mean±SEM.
*P < 0.01 (t test, control vs. Rx).

of 115%, whereas the high-dose group increased by a mean of 174%.

Intestinal phosphate absorption. In the low-dose group there was a mean improvement in net intestinal phosphate absorption of 49% (+60 mg/d, P < 0.05) (Table V and Fig. 2). In the high-dose group the mean decreased 35%, owing primarily to patient 4. By analysis of variance using the individual collection

---

**Figure 3** Blood and urine $^{47}$Ca kinetic data for patients given low (A) and high doses (B) of hPTH-(1–34) during control and treatment studies. ○, The control study; ●, the treatment study. All data points are the mean of the patients in each group. Note that the upper panels represent blood and urine $^{47}$Ca specific activity, whereas the lower panels represent cumulative fraction of the injected $^{47}$Ca excreted in the urine.
periods, this change in the high-dose group was not significant. Patient 4 had unusually inefficient intestinal phosphate absorption during the control periods, which was not associated with any other abnormality in phosphate metabolism or intestinal function.

**Balance.** In all three patients given the lower dose, phosphate balance improved (mean + 85%, mean increase 74 mg/d) (Table V and Fig. 2). This was statistically significant (P < 0.01) when all individual periods were used in the analysis of variance. This improvement was primarily due to an increase in intestinal phosphate absorption.

Again these effects of the two different peptide doses on balance were different (P < 0.01).

Phosphate balance became negative in those patients receiving the high dose of hPTH-(1-34) (P < 0.01). The mean decrease in balance of 245% over control represented a mean of 178 mg phosphate/d. This resulted both from a decrease in intestinal phosphate absorption of 91 mg/d, and an increase in urinary phosphate excretion of 87 mg/d.

**Magnesium metabolism**

The mean levels of fasting plasma magnesium were significantly lowered (P < 0.01) by hPTH-(1-34) (1.91±0.02 control vs. 1.75±0.02 mg/dl treatment) with both doses and was significantly lowered (P < 0.01) in all patients except patient 2, who showed no change.

There were no consistent differences in the mean urinary excretion of magnesium, net intestinal magnesium absorption, and magnesium balance. The lowered plasma magnesium levels were perhaps due to renal losses too small to be appreciated.

**DISCUSSION**

Several results of the present study are pertinent with regard to the long-term use of this agent to alter bone metabolism in patients with osteoporosis.

In the present study, patients receiving the higher dose (750 U) of hPTH-(1-34) had a significant increase in urinary calcium and phosphate excretion, not accompanied by an increase in net intestinal absorption of calcium and phosphate. In two of three patients, the calcium and phosphate balances became significantly worse; this observation implies that this dose of hPTH-(1-34) causes either net bone breakdown or a primary imbalance between intestinal absorption and urinary excretion of phosphate and calcium. In addition, because hPTH-(1-34) administration caused greater changes in phosphate balance in patients 4 and 5 than would be expected if the phosphate were lost
entirely from bone (calcium: phosphate ratio 0.9:1 in patient 4 and 1.2:1 in patient 5), these two patients probably also lost phosphate from soft tissue stores. Parathyroid hormone excess also causes loss of bone mineral and of soft tissue phosphate in patients with primary hyperparathyroidism (48). The inference of net bone breakdown in the patients receiving 750 U hPTH-(1–34) is reinforced by several independent lines of evidence. In patient 5, urinary hydroxyproline excretion rose above the normal range during treatment with this dose of hPTH-(1–34). In patients 4 and 5 there was a significant increase in plasma calcium concentration, and patient 5 became transiently hypercalcemic 6 h after the injection of hormone. In patients 4 and 5 the serum phosphate concentration rose during the 4 wk of hormone administration with 750 U hPTH-(1–34) despite a reduction in the Tmp/GFR. These data, taken together, further suggest bone mineral loss.

In contrast to these effects, patients receiving the lower dose (450 U) of hPTH-(1–34) showed no increase in urinary phosphate excretion and only a small increase in urinary calcium excretion, and their calcium and phosphate balances either improved or remained essentially unchanged. Such improvements were largely related to improved intestinal calcium and phosphate absorption. These improvements occurred without the production of hypercalcemia or hypophosphatemia and probably indicate net bone uptake of both ions. The peptide apparently has a narrow range of dose dependence. A biphasic dose-response relation has also been seen in various animal studies (49), and suggests induction of some counter-regulatory effect or effects, the nature of which is unknown.

A surprising finding in this study was the lack of a more marked effect of hPTH-(1–34) on intestinal calcium and phosphate absorption in view of the known stimulatory effect of parathyroid hormone on the renal 25-hydroxyvitamin-D-1-hydroxylase system (50–52) and the high intestinal calcium absorption reported in many patients with primary hyperparathyroidism (53). Birge et al. (54) showed in one patient that there were delayed effects of parathyroid hormone on intestinal calcium absorption. Recent evidence has indicated the need for prolonged (many hours) elevation of parathyroid hormone in blood before serum levels of 1,25-dihydroxyvitamin D₃ increase (55). Blood levels of hPTH-(1–34) in our patients after a single subcutaneous dose, estimated by radioimmunoassay, suggest rapid absorption and high but transient blood levels of the peptide. This may explain why the peptide failed to increase intestinal calcium absorption consistently. More frequent administration of parathyroid hormone may be needed to increase intestinal calcium and phosphate absorption. Patients with primary hyperparathyroidism have increased phosphate absorption (56, 57), whereas the acute administration of parathyroid hormone to normal subjects does not alter radio phosphorus absorption (58).

A second important aspect of this study concerns the time-related effects of this hormone on bone. There are almost no other available data concerning the early effects of synthetic hPTH-(1–34) or any other parathyroid hormone fragment on bone turnover in humans. Some studies have evaluated bone mineral metabolism in patients treated chronically with hPTH-(1–34) for 6 mo (21–24). In all patients in the present study, hPTH-(1–34) accelerated turnover of the labile calcium pool, as shown by a decrease in the mean transit time of the exchangeable pool and a decrease in the size of this pool. This finding shows that as little as several weeks of daily hPTH-(1–34) therapy accelerates bone calcium turnover. This acceleration was associated with a more rapid decrease in blood and urine ⁴⁷Ca specific activity plus an increase in renal excretion of ⁴⁷Ca as a result of hPTH-(1–34) in both groups. The⁴⁷Ca kinetic study did not show any significant changes in bone accretion after short-term hPTH-(1–34) administration, a finding substantiated by the lack of any change in alkaline phosphatase activity. These changes are in marked contrast to those seen in another study after more prolonged hPTH-(1–34) administration (47). In that study, hPTH-(1–34) administration for 6 mo accelerated the skeletal ⁴⁷Ca turnover time threefold or more and markedly stimulated skeletal ⁴⁷Ca accretion.

A related example of the time-dependent response to hPTH-(1–34) administration is provided by the urinary calcium measurements (Fig. 1). During the first 2 wk of hormone administration, there is a definite increase in renal calcium excretion, which then appears to reach a plateau for the remainder of the study.

An interesting finding was the marked increase in urinary phosphate excretion during the 3 h after hPTH-(1–34) administration as compared to the 24-h collection. It appears that there was a burst of phosphate excretion during this time and then a reduced phosphate excretion during the remainder of the day in all patients except 4 and 5, so that the 24-h excretion was not markedly different from control. Phosphate excretion was not reduced in patients 4 and 5 for the remainder of the day. Total phosphate excretion increased significantly in these subjects owing at least in part to net bone breakdown.

Although reports indicate an increase in bone turnover and in trabecular bone volume in subjects treated chronically with hPTH-(1–34) (21–24), the failure of intestinal calcium absorption and calcium balance to increase consistently would seem to limit the long-term anabolic effects of this agent in patients with osteoporosis. Further study of the mechanism of the effect of parathyroid hormone on the skeleton as well as the
effect of other concomitantly administered agents to increase intestinal absorption or reduce bone resorption would seem to be indicated if the therapeutic potential of this hormone in osteoporosis is to be meaningfully tested.

ACKNOWLEDGMENTS

We wish to thank Cynthia Benney and Don Lans for analyzing the various samples, and all the personnel of the Clinical Research Center for their painstaking efforts during the balance studies. We would also like to thank Dr. Jonathan Reeve for helping to analyze the 45Ca kinetic studies, Dr. Pierre Meunier and Dr. Claude Edouard for the histomorphometric analyses, and Linda Gardner for her secretarial help.

This work was supported by a gift from The Lida R. and Charles H. Tompkins Foundation, National Institutes of Health grants AM04501, AM17930, and RR01066, and National Aeronautics and Space Administration contract NAS9-15204.

REFERENCES


