Altered diurnal regulation of blood ionized calcium and serum parathyroid hormone concentrations during parenteral nutrition\textsuperscript{1–3}

William G Goodman, Sudipta Misra, Johannes D Veldhuis, Anthony A Portale, He-Jing Wang, Marvin E Ament, and Isidro B Salusky

ABSTRACT
Background: Little is known about parathyroid gland function in patients receiving total parenteral nutrition (TPN).
Objective: Our objective was to determine whether parathyroid gland function is abnormal in TPN recipients.
Design: Six patients with a mean (±1 SD) age of 45.5 ± 8.0 y who had been receiving TPN for 18.7 ± 2.8 y underwent bone biopsy, bone mass measurements with dual-energy X-ray absorptiometry, and dynamic tests of parathyroid gland function. Diurnal variations in blood ionized calcium (iCa\textsuperscript{2+}) and serum parathyroid hormone (PTH) concentrations were also assessed.
Results: Results were compared with those of healthy volunteers.
Results: Bone mass and bone formation were subnormal in all patients. Basal serum PTH concentrations were moderately higher in the TPN recipients than in healthy volunteers, and values obtained every 30 min over 24 h were significantly higher (P < 0.001) in TPN recipients (5.0 ± 0.9 pmol/L) than in healthy volunteers (2.6 ± 0.6 pmol/L). The percentage increase in serum PTH during citrate-induced hypocalcemia was lower in the TPN recipients, consistent with secondary hyperparathyroidism. Evening infusions of calcium-containing TPN eliminated the nocturnal rise in serum PTH, increased the amplitude of change for iCa\textsuperscript{2+} and PTH over 24 h, increased the orderliness of change for iCa\textsuperscript{2+} and PTH as measured by approximate entropy (ApEn), and enhanced the synchrony of change between iCa\textsuperscript{2+} and PTH. Treatment for 10 d with calcium-free TPN restored the nocturnal rise in serum PTH and increased ApEn for PTH. ApEn for iCa\textsuperscript{2+} remained low, suggesting that a component of nutrient solutions, but not calcium per se, enhances the regularity of PTH release in TPN recipients.

KEY WORDS Parathyroid hormone, total parenteral nutrition, TPN, metabolic bone disease, hyperparathyroidism, diurnal variation, adults, bone biopsy, blood ionized calcium, bone mass

INTRODUCTION
Mineral metabolism is abnormal in patients receiving total parenteral nutrition (TPN); many of these patients have clinical or biochemical evidence of metabolic bone disease and osteoporosis (1–6). Despite considerable previous work, the factors responsible for metabolic bone disease are poorly understood (5). Bone aluminum accumulation, disturbances in vitamin D metabolism, and alterations in secretion of parathyroid hormone (PTH) have each been implicated in the pathogenesis of bone disease in TPN recipients (7–9). Little is known, however, about parathyroid gland function in patients receiving long-term TPN.

Serum PTH concentrations were reported to be normal, high, and low in long-term TPN recipients (7–10). Low values are generally attributed to the inhibition of PTH release by calcium-containing TPN solutions (9), which may account for the reductions in bone formation and turnover that characterize patients receiving TPN (6, 10). Detailed assessments of parathyroid gland function in TPN recipients have not been made, and there is little information about the effect on basal serum PTH concentrations of varying the calcium content of TPN solutions. Additionally, the temporal relation between daily nutrient infusions and fluctuations in blood ionized calcium (iCa\textsuperscript{2+}) and serum PTH concentrations throughout the day has not been examined despite evidence that calcium excretion in urine is greater when intermittent rather than continuous intravenous infusions are used (11, 12).

Several studies indicated that diurnal variations in serum PTH concentrations influence bone metabolism (13, 14) and that nocturnal increases in PTH secretion favorably affect bone mass (13, 14). It has not been determined, however, whether the normal diurnal variations in iCa\textsuperscript{2+} and serum PTH are preserved in

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patients receiving daily intravenous infusions of calcium-containing nutrient solutions (13, 15, 16). The current study was therefore undertaken to better characterize the fluctuations in iCa\textsuperscript{2+} and serum PTH concentrations throughout the day and to further examine the secretory behavior of the parathyroid glands in patients receiving long-term TPN.

SUBJECTS AND METHODS

Subjects

Six patients receiving long-term TPN at the University of California Los Angeles Medical Center were evaluated. The mean (±1 SD) age of the patients was 45.5 ± 8.0 y (range: 33–57 y); 3 were women and 3 were men. The duration of TPN before the study was 18.7 ± 2.8 y (range: 15–22 y). All TPN recipients who agreed to a bone biopsy and an overnight hospital stay in the General Clinical Research Center (GCRC) were eligible to participate in the study; participants were not selected on the basis of clinical or biochemical evidence of bone disease.

All patients received daily infusions of 1.0–1.5 L TPN solution over 6 h, starting at 2100. TPN solutions contained 8.5% amino acids, 50% dextrose, a multivitamin preparation providing 200 IU vitamin D, and various trace elements, including zinc, copper, and selenium. The calcium and phosphorus contents of the TPN solutions varied among patients; the amount of calcium infused ranged from 200 to 600 mg/d and the amount of elemental phosphorus ranged from 240 to 775 mg/d, or 7.7–25 mmol/d. The ratio of calcium to phosphorus in TPN solutions averaged 0.61, ranging from 0.32 to 1.08. Patients were not given supplemental vitamin D sterols such as 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D orally or parenterally. None of the patients had been treated with phenobarbital or phenytoin.

All patients received TPN for the management of short-bowel syndrome; causes of the disorder included mesenteric vein thrombosis in 2 patients, radiation enteritis in 1 patient, Crohn disease in 2 patients, and midgut volvulus with strangulation in 1 patient. No study participant had a malignant tumor, granulomatous disease, or any other systemic disorder known to affect calcium, phosphorus, or bone metabolism. Of the 2 patients with Crohn disease, 1 had not received corticosteroids for >15 y and the other was last treated with prednisone >5 y before the study began. All studies were approved by the UCLA Human Subjects Protection Committee and written informed consent was obtained from each study participant.

Protocol

Patients were evaluated during 2 separate 2-d admissions to the GCRC; parathyroid gland function was studied before and 10 d after calcium was removed from the TPN solutions. During each GCRC admission and throughout the study, TPN was continued by using the same schedule of evening infusions that was regularly used by each patient for ongoing therapy.

For the initial GCRC admission, patients were evaluated while receiving calcium-containing TPN solutions. The amount of calcium provided to each patient was the same as that used regularly for long-term management. On the first day of the study, the secretory response of the parathyroid glands was evaluated by gradually lowering iCa\textsuperscript{2+} concentrations by ≥0.2 mmol/L during 2-h intravenous infusions of sodium citrate (17, 18). The initial rate of sodium citrate infusion was 28 mg·kg\textsuperscript{-1}
·h\textsuperscript{-1}, which was increased in stepwise increments every 20 min to achieve a rate of 118 mg·kg\textsuperscript{-1}
·h\textsuperscript{-1} during the final 20 min of 2-h infusions (17, 18). Blood samples for measurements of iCa\textsuperscript{2+} and serum PTH concentrations were obtained 30, 15, and 0 min before; at 2-min intervals during the first 10 min; and at 3-min intervals during the second 10 min of each infusion. Thereafter, samples were collected every 10 min for the remainder of the 120-min infusions (17, 18). All assessments were made in the morning ≥6 h after nocturnal TPN infusions were completed.

iCa\textsuperscript{2+} concentrations were monitored throughout the 2-h study by using a calcium-specific electrode (Radiometer ICA-II, Copenhagen); blood samples were collected anaerobically and measurements were obtained immediately thereafter. Serum samples for PTH measurements were separated by centrifugation (2000 × g for 10 min at 4 °C) immediately after collection, snap frozen on solid carbon dioxide, and stored at −70 °C until assayed (18). iCa\textsuperscript{2+} concentrations were monitored after citrate infusions were stopped until values returned to baseline concentrations. None of the patients received TPN infusions during the administration of sodium citrate. After sodium citrate infusions were completed, bone mass was measured by dual-energy X-ray absorptiometry and an iliac crest bone biopsy was performed.

The next day, blood samples were collected every 30 min for 24 h to characterize the diurnal variations in iCa\textsuperscript{2+} and serum PTH concentrations during TPN by using calcium-containing solutions (13). Serial blood samples were obtained through an indwelling venous catheter. iCa\textsuperscript{2+} concentrations were measured immediately after samples were obtained; serum samples were prepared and stored as described previously for subsequent measurements of PTH concentrations. Half-hourly blood collections were started at 0900 and the final blood sample was obtained at 0900 the next morning. Patients were given free access to water during the 24 h of observation, but no additional nourishment other than that delivered by TPN was provided. As noted previously, the schedule of TPN infusions was kept the same as that used by each subject during home parenteral nutrition.

After the baseline studies, the patients were administered TPN solutions containing no added calcium for 10 d. The composition of each subject’s nutrient solution was otherwise the same as that used for ongoing therapy. The time of day at which nutrient infusions began and the duration of each infusion remained unchanged; thus, the schedule of nutrient infusions during the 10 d of calcium-free TPN administration corresponded in each patient to that used both before the study and during the initial assessment using calcium-containing TPN solutions.

After 10 d of treatment with calcium-free TPN solutions, patients were readmitted to the GCRC and the response to citrate-induced hypocalcemia was reevaluated. The next day, iCa\textsuperscript{2+} and serum PTH concentrations were measured every 30 min for 24 h as described previously. At the conclusion of the study, patients resumed their previous TPN regimen with calcium-containing solutions.

The results obtained during 2-h sodium citrate infusions in TPN recipients were compared with data obtained by using the same study protocol in 20 volunteers with normal renal and parathyroid gland function, as reported elsewhere (18). Results for iCa\textsuperscript{2+} and serum PTH measured at 30-min intervals over 24 h for patients receiving either calcium-containing or calcium-free TPN were compared with data collected from a separate group of 10 healthy adult male volunteers aged 37.6 ± 5.5 y (19).
Bone biopsy and quantitative histomorphometry of bone

TPN recipients underwent an iliac crest bone biopsy after double tetracycline labeling with a 14-d interlabel interval (20). Quantitative histomorphometry of bone was done as described previously on 5-μm plastic-embedded sections of nondecalcified bone stained according to the modified Goldner technique (20). Tissue sections were examined by light microscopy with a Leitz Dialux microscope (Leitz, Wetzlar, Germany) (20, 21). Five-micrometer sections were also stained by using the aurine tricarboxylic acid method for aluminum determinations (20, 21). Ten-micrometer sections of bone were mounted unstained in 10% glycerol to enable tetracycline labels to be visualized by using epifluorescence microscopy. Microscopic images were digitized by using a SummaGrid digitizer tablet connected to a desktop computer (20, 21).

All bone histomorphometry results represent measurements in 2 dimensions, and the terminology of the Nomenclature Committee of the American Society for Bone and Mineral Research was used for the presentation of results (22). Values reported include the trabecular bone area (Bar/TA), expressed as a percentage of total tissue area; the osteoid area (Oar/Bar), expressed as a percentage of trabecular bone area; the osteoid perimeter (OPm/TbPm), expressed as a percentage of the total trabecular perimeter; the osteoid seam width (OWi), expressed in μm; the eroded bone perimeter (EPm/TbPm), expressed as a percentage of the total trabecular perimeter characterized by scalloped resorption lacunae; the double-tetracycline-labeled surface (dLS), expressed as a percentage of the trabecular bone perimeter; and the bone formation rate (BFR/TA), expressed as μm²·mm⁻²·d⁻¹. The results obtained in TPN recipients were compared with reference data for 29 subjects who had no evidence of metabolic bone disease, as reported previously (23).

Bone mass measurements

Measurements of bone mass were obtained at the lumbar spine and at the femoral neck by using a Hologic 2000 dual-energy X-ray absorptiometer (Hologic Inc, Waltham, MA). Results for the spine represent measurements obtained from the first through the fourth lumbar vertebrae; values at the hip reflect measurements made at the midpoint of the femoral neck. The results for bone mass (in g/cm²) at each skeletal site were also made at the midportion of the femoral neck. The first through the fourth lumbar vertebrae; values at the hip reflect results for bone mass (in g/cm²) at each skeletal site were also made at the midportion of the femoral neck.

Biochemical measurements

Total serum calcium, phosphorus, and alkaline phosphatase concentrations were measured by using methods described elsewhere (18, 20). iCa²⁺ concentrations were measured in duplicate by using calcium-specific electrodes (Radiometer II, Copenhagen; or Nova 8 Analyzer, Waltham, MA). Serum PTH concentrations were measured in duplicate by using an immunoradiometric assay for the intact 84 amino acid hormone (24). Serum 25-hydroxyvitamin D concentrations were measured by competitive protein-binding assay (25) and serum 1,25-dihydroxyvitamin D concentrations were measured by radioreceptor assay (26).

Statistical analysis

All data are expressed as means (±1 SD). Comparisons among 3 or more groups were done by using one-way analysis of variance (ANOVA) with Scheffe’s multiple comparison procedures (27). Paired t tests were used to compare results obtained from individual patients studied before and after calcium was removed from the TPN solutions (27); unpaired t tests were used to compare baseline bone mass measurements with age- and sex-appropriate normative values (27).

Changes in serum PTH concentrations during 2-h sodium citrate infusions were evaluated by repeated-measures ANOVA and by nonlinear curve-fitting procedures; variations in iCa²⁺ and serum PTH concentrations over 24 h were evaluated by cosinor analysis (27–29). For cosinor analysis, the goodness of fit to a sinusoidal curve was determined for data collected every 30 min over 24 h in each study subject by using a nonlinear model of the following form: y = A cos(k × t + w) + B, where B is the grand mean of all values, A is the difference between the value at each sampling interval and the overall mean, k is cycle length, t is time, and w is the offset of the start of the cycle from a fixed time referent. The amplitude of the difference between peak and nadir values over 24 h was measured for iCa²⁺ and for serum PTH. Comparisons among groups for fitted mean values with 95% CIs were made by using the Monte Carlo support plane procedure assuming highly correlated variables with asymmetric variance spaces or bootstrap methods (29–32).

The relation between iCa²⁺ and serum PTH concentrations over 24 h was examined by cross-correlational analysis in healthy volunteers and in patients receiving TPN with or without calcium (33). The regularity of change, or orderliness, of PTH and iCa²⁺ concentrations throughout the day was measured by using the approximate entropy (ApEn) statistic (34). The synchrony between iCa²⁺ and PTH was assessed by the cross-approximate entropy statistic (X-ApEn) (35). Comparisons among groups for each of these variables were made by using ANOVA.

RESULTS

Bone mass measurements

Bone mass was lower than normal in all 6 patients receiving long-term TPN as assessed by dual-energy X-ray absorptiometry. The z scores for bone mineral content averaged −1.97 ± 0.59 at the lumbar spine and −2.17 ± 0.98 at the femoral neck. Individual values ranged from −1.33 to −2.78 and from −1.20 to −3.28 at the spine and the hip, respectively. Thus, bone mass at each site fell at ≥1 SD below the mean normal value for subjects of the same age and sex in all patients receiving TPN.

Bone histomorphometry

The BFR/TA was subnormal in all 6 long-term TPN recipients when assessed by using the double-tetracycline-labeling technique with quantitative bone histomorphometry, and BAr/TA was below the lower limit of normal in 2 of them (Table I). Average values for the extent of OPm/TbPm, for EPm/TbPm, and for OWi were greater than normal. However, the static histologic indexes of bone formation varied considerably among patients. In 2 patients, OPm/TbPm and OWi were markedly elevated and the uptake of tetracycline into bone was low, indicating osteomalacia. In the remaining 4 patients, OPm/TbPm was only mildly elevated and OWi was within the normal range. Such findings are most consistent with a low-turnover, adynamic lesion of bone (ie, low-turnover osteoporosis) without evidence of impaired mineralization. None of the patients had evidence of bone aluminum deposition as judged by histochemical staining methods.
Bone histomorphometric measurements in patients receiving long-term total parenteral nutrition

<table>
<thead>
<tr>
<th>Patient</th>
<th>OAr/Bar</th>
<th>OPm/TbPm</th>
<th>OWi</th>
<th>EPm/TbPm</th>
<th>dLS</th>
<th>BFR/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.7%</td>
<td>15.6%</td>
<td>67.4%</td>
<td>14.5%</td>
<td>4.4%</td>
<td>0.5%</td>
</tr>
<tr>
<td>2</td>
<td>12.9%</td>
<td>4.4%</td>
<td>21.0%</td>
<td>9.4%</td>
<td>0.9%</td>
<td>0.9%</td>
</tr>
<tr>
<td>3</td>
<td>18.5%</td>
<td>4.6%</td>
<td>23.9%</td>
<td>12.5%</td>
<td>2.7%</td>
<td>1.0%</td>
</tr>
<tr>
<td>4</td>
<td>17.9%</td>
<td>22.7%</td>
<td>85.1%</td>
<td>14.2%</td>
<td>4.5%</td>
<td>0.0%</td>
</tr>
<tr>
<td>5</td>
<td>14.6%</td>
<td>4.9%</td>
<td>22.7%</td>
<td>12.7%</td>
<td>1.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>6</td>
<td>16.3%</td>
<td>8.9%</td>
<td>37.5%</td>
<td>12.9%</td>
<td>3.7%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Reference values (n = 29)

<table>
<thead>
<tr>
<th>Reference values</th>
<th>OAr/Bar</th>
<th>OPm/TbPm</th>
<th>OWi</th>
<th>EPm/TbPm</th>
<th>dLS</th>
<th>BFR/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>x ± 1 SD</td>
<td>15.7 ± 2.3%</td>
<td>10.2 ± 7.5%</td>
<td>42.9 ± 27.0%</td>
<td>12.7 ± 1.8%</td>
<td>2.9 ± 1.5%</td>
<td>0.4 ± 0.5%</td>
</tr>
<tr>
<td>Range</td>
<td>14.6–26.9</td>
<td>0.2–5.8</td>
<td>4.3–31.7</td>
<td>4.1–13.2</td>
<td>0.5–4.3</td>
<td>1.6–15.9</td>
</tr>
</tbody>
</table>

Biochemical determinations

When initially evaluated during administration of the calcium-containing TPN solutions, serum total calcium concentrations were significantly lower than normal, whereas serum phosphorus values did not differ significantly from normal (Table 2). Although the mean serum concentration of 25-hydroxyvitamin D was lower than normal, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were within the normal range in all 6 TPN recipients. In contrast, serum PTH concentrations at 0900, which represent samples obtained 6 h after the TPN infusions were completed, were higher in TPN recipients than in healthy volunteers. The average of serum PTH concentrations obtained every 30 min over 24 h was also substantially greater in patients receiving calcium-containing TPN than in healthy volunteers. Calcium excretion in urine ranged from 113 to 450 mg/d in TPN recipients and daily renal calcium excretion correlated with the amount of calcium added to TPN solutions (r = 0.79).

After administration of calcium-free TPN for 10 d, serum calcium and phosphorus concentrations tended to decrease slightly but serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations remained unchanged. Calcium excretion in urine fell to < 2.5 mmol/d in 3 of 6 patients. Serum PTH concentrations at 0900 rose slightly, but values exceeded 10 pmol/L sometime during the day in 2 of 6 patients when measurements were obtained every 30 min over 24 h (see below). As such, the mean of half-hourly serum PTH concentrations was greater in patients given calcium-free TPN solutions than when the same patients were assessed while receiving calcium-containing TPN.

Assessments of parathyroid gland function

During 2-h infusions of sodium citrate, serum PTH concentrations increased rapidly and remained elevated for the full 120 min of each infusion (Figure 1). Maximum serum PTH concentrations were achieved within the first 10–20 min in TPN recipients and in subjects with normal renal and parathyroid gland function. Despite equivalent rates of change in iCa2+ during citrate infusions, the percentage increase in serum PTH during citrate infusions was less in patients receiving calcium-containing TPN than in healthy volunteers.

Serum biochemical determinations in patients receiving long-term total parenteral nutrition (TPN) with and without calcium

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers (n = 10)</th>
<th>TPN with calcium (n = 6)</th>
<th>TPN without calcium (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total calcium (mmol/L)</td>
<td>2.38 ± 0.10</td>
<td>2.13 ± 0.11²</td>
<td>2.03 ± 0.21²</td>
</tr>
<tr>
<td>Blood ionized calcium (mmol/L)</td>
<td>1.20 ± 0.03</td>
<td>1.24 ± 0.03²</td>
<td>1.19 ± 0.06³</td>
</tr>
<tr>
<td>24-h mean</td>
<td>1.19 ± 0.03</td>
<td>1.23 ± 0.03²</td>
<td>1.22 ± 0.04³/⁴</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>2.1 ± 0.5</td>
<td>5.2 ± 3.4²</td>
<td>6.2 ± 1.6²</td>
</tr>
<tr>
<td>24-h mean</td>
<td>2.6 ± 0.6</td>
<td>5.0 ± 0.9³</td>
<td>6.1 ± 0.6⁴/⁵</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>1.23 ± 0.10</td>
<td>1.26 ± 0.23</td>
<td>1.16 ± 0.16</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (nmol/L)</td>
<td>53 ± 7</td>
<td>39 ± 7²</td>
<td>46 ± 12</td>
</tr>
<tr>
<td>Serum 1,25-dihydroxyvitamin D (nmol/L)</td>
<td>77 ± 19</td>
<td>61 ± 34</td>
<td>87 ± 39</td>
</tr>
<tr>
<td>Urinary calcium excretion (mmol/d)</td>
<td>42.8 ± 24.0</td>
<td>64.5 ± 34.8</td>
<td>30.8 ± 17.5²</td>
</tr>
</tbody>
</table>

¹ x ± 1 SD; n = 6.
²³ Significantly different from healthy volunteers (unpaired t test): ² P < 0.01, ³ P < 0.001, ⁴ P < 0.05.
⁵ Significantly different from TPN with calcium, P < 0.01 (unpaired t test).
The variations in serum PTH and iCa\textsuperscript{2+} concentrations throughout the day differed markedly from normal in patients receiving long-term TPN. Compared with those of healthy volunteers (Figure 2), iCa\textsuperscript{2+} concentrations were progressively higher and serum PTH values were substantially lower during evening infusions of calcium-containing TPN solutions (Figure 2). The amplitude of change for iCa\textsuperscript{2+} and serum PTH concentrations over 24 h was therefore greater than normal in patients receiving calcium-containing TPN (Table 3). In patients maintained on calcium-free TPN for 10 d, iCa\textsuperscript{2+} concentrations decreased during the late evening and early morning hours, and these changes were associated with reciprocal increases in serum concentrations of PTH (Figure 2, bottom panel). Although the characteristic nocturnal rise in serum PTH concentrations was restored in patients receiving calcium-free TPN, the amplitude of change throughout the day for iCa\textsuperscript{2+} and serum PTH remained greater than normal (Figure 2, bottom panel) (Table 3).

There was a negative cross-correlation between the concentrations of iCa\textsuperscript{2+} and serum PTH in samples obtained simultaneously in healthy volunteers when these were assessed every 30 min for 24 h (Figure 3, top panel). There was a somewhat stronger negative cross-correlation between iCa\textsuperscript{2+} and PTH in patients maintained on calcium-containing TPN (Figure 3, middle panel). This inverse relation was sustained for PTH values that lagged behind iCa\textsuperscript{2+} concentrations for 30 min and for PTH concentrations that preceded iCa\textsuperscript{2+} values by as much as 90 min (Figure 3, middle panel). When patients were given calcium-free TPN, the negative cross-correlation between iCa\textsuperscript{2+} and serum PTH concentrations extended over lag intervals ranging from −120 to 120 min (Figure 3, bottom panel).

Measurements of ApEn for iCa\textsuperscript{2+} and serum PTH concentrations over 24 h showed considerable irregularity of change throughout the day in healthy volunteers (Figure 4). In contrast, patients maintained on calcium-containing TPN had the lowest ApEn values for iCa\textsuperscript{2+} and PTH, reflecting greater regularity of change in these 2 variables over time. X-ApEn values were also lower in patients given calcium-containing TPN than in healthy volunteers, indicating greater synchrony between the changes in iCa\textsuperscript{2+} and serum PTH in patients receiving conventional TPN solutions (Figure 5). After 10 d of calcium-free TPN, ApEn for PTH increased to values that did not differ significantly from those measured in healthy volunteers (Figure 4); X-ApEn values for iCa\textsuperscript{2+} and PTH also rose after calcium was removed from the TPN solutions (Figure 5). ApEn for iCa\textsuperscript{2+} remained low, however, and the results for patients receiving calcium-free TPN did not differ significantly from those obtained during the administration of calcium-containing TPN solutions (Figure 4).

<table>
<thead>
<tr>
<th>TABLE 3</th>
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</thead>
<tbody>
<tr>
<td>Amplitude of change for blood ionized calcium and serum parathyroid hormone (PTH) concentrations in healthy volunteers and in patients receiving long-term total parenteral nutrition (TPN) with and without calcium</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Blood ionized calcium (mmol/L)</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} ± 1 SD; \textit{n} = 6. Amplitude of change refers to the difference between the maximum and minimum values observed over 24 h.

\textsuperscript{2} Significantly different from healthy volunteers, \textit{P} < 0.01 (one-way ANOVA).

\textsuperscript{3} Significantly different from TPN with calcium, \textit{P} < 0.05 (one-way ANOVA).
DISCUSSION

The results of the current investigation confirm data reported previously indicating that bone mass is reduced in adults receiving long-term TPN (12, 36, 37). Bone mineral content at the lumbar spine and the femoral neck was > 1 SD below age- and sex-appropriate reference values in all TPN recipients as measured by dual-energy X-ray absorptiometry. The $z$ scores for bone mass at the femoral neck were lower than those for bone mass at the lumbar spine, suggesting that the bone loss associated with long-term TPN disproportionately affects cortical bone.

The number of patients in the current study was small, and bone biopsy was done primarily to enable documentation of the specific skeletal lesion in TPN recipients who were undergoing detailed assessments of parathyroid gland function. Two patients had osteomalacia and 4 patients had low-turnover lesions of bone without evidence of impaired mineralization; these results are consistent with data reported by others (6, 10, 36, 38). The factors responsible for these skeletal changes are not, however, readily apparent. None of the TPN recipients had bone aluminum deposition, which can lead to either osteomalacia or low-turnover lesions of bone (38–40). Serum calcium and phosphorus concentrations were generally well maintained in all study participants, and none of the patients had been given phenobarbital or phenytoin, agents that can alter vitamin D metabolism and cause osteomalacia (41). Nevertheless, 2 of 3 women were postmenopausal, and both of these had low-turnover skeletal lesions without evidence of impaired mineralization, changes that are consistent with osteoporosis due to estrogen deficiency (42). Androgen deficiency may have contributed to reductions in bone formation and turnover in 2 of 3 male patients, but serum testosterone and gonadotrophin concentrations were not available (43). The role of alterations in sex steroid production or metabolism in TPN-related bone disease therefore remains unclear.

Although the serum concentrations of vitamin D metabolites fell within the lower range of normal in all 6 study patients, biochemical evidence of altered bone and mineral metabolism is found in a substantial proportion of hospitalized patients when 25-hydroxyvitamin D concentrations are near the lower limit of normal (44). Inadequate vitamin D nutrition may contribute, therefore, to the development of osteomalacia or mild secondary hyperparathyroidism in some long-term TPN recipients.

Because exogenous calcium infusions can lower serum PTH concentrations and reduce bone formation by suppressing PTH secretion, the current study was designed to provide more detailed information about parathyroid gland function in patients receiving long-term TPN. The results eliminate functional hypoparathyroidism as an explanation for diminished bone formation and osteoblastic activity in patients given TPN solutions containing currently recommended amounts of calcium. Indeed, serum PTH concentrations at 0900 and the average of concentrations obtained every 30 min over 24 h were substantially higher in TPN recipients than in subjects with normal renal and parathyroid gland function. Such findings indicate that PTH secretion is modestly elevated under baseline conditions in patients receiving intermittent infusions of calcium-containing TPN solutions, despite the fact that iCa$^{2+}$ concentrations in the morning fall within the normal range. Moderate parathyroid gland hyperplasia could account for this change, and intermittent reductions in iCa$^{2+}$ calcium during the day, as documented in the current study, may provide the stimulus for glandular hyperplasia in TPN recipients. The finding that bone formation was subnormal despite moderate increases in serum PTH concentrations also suggests that the bone of patients receiving long-term TPN is relatively resistant to the actions of PTH, as suggested previously by de Vernejoul et al (10).

The response to citrate-induced hypocalcemia provides further evidence that patients receiving long-term TPN have mild secondary hyperparathyroidism (18). When expressed as a percent-

\[ \text{FIGURE 3. Cross-correlations between blood ionized calcium and serum parathyroid hormone concentrations in 10 volunteers with normal renal and parathyroid gland function (top panel) and in 6 patients receiving long-term total parenteral nutrition (TPN) with (middle panel) and without (bottom panel) calcium. Symbols (△) represent medians and lines depict ranges of the cross-correlational coefficients. *Significant difference between 0- and 10-d measurements, } P < 0.05 \ (\text{Kolmogorov-Smirnov test}). \]
Ionized calcium (\(\text{iCa}^{2+}\)) with and without calcium. * Significantly different from healthy volunteers, \(P < 0.05\).

In addition to higher basal serum PTH concentrations and reductions in hypocalcemia-induced increases in serum PTH concentrations, the normal diurnal variations in \(\text{iCa}^{2+}\) and serum PTH were disrupted in long-term TPN recipients. The orderliness of PTH release was enhanced in patients receiving nocturnal infusions of calcium-containing TPN solutions, and this abnormality was only partially corrected by removing calcium from the TPN solutions. Such findings suggest that the timing and the amount of calcium provided during parenteral nutrition affects several intrinsic components of parathyroid gland function.

Cross-correlational analysis and the statistical measures ApEn and X-ApEn were used to further assess selected features of PTH secretion in patients receiving long-term TPN. ApEn quantifies the orderliness of variation in values over time (34). Measurements of ApEn for \(\text{iCa}^{2+}\) and serum PTH concentrations in healthy volunteers showed considerable physiologic irregularity in the fluctuation of both variables throughout the day. Compared with healthy volunteers, patients given calcium-containing TPN solutions had lower ApEn values for both serum PTH and \(\text{iCa}^{2+}\); such findings indicate that regularly scheduled nocturnal TPN infusions increase the orderliness of change in \(\text{iCa}^{2+}\) and PTH concentrations, perhaps by delivering relatively large amounts of calcium intravenously over a few hours. In contrast, dietary calcium absorbed from the gastrointestinal tract in healthy subjects enters the extracellular fluid more slowly after the ingestion of meals that are typically separated by many hours.

Removal of calcium from the TPN solutions increased ApEn for PTH toward normal but ApEn for \(\text{iCa}^{2+}\) remained low; indeed, measurements of ApEn for \(\text{iCa}^{2+}\) did not differ significantly before and after calcium was removed from the TPN solutions. The disparity between ApEn values for \(\text{iCa}^{2+}\) and PTH in patients receiving calcium-free TPN suggests that a factor associated with TPN, but not with calcium per se, accounts for the increased regularity of PTH release during TPN using calcium-containing TPN solutions. Although the mechanism responsible for this disturbance is unknown, changes in osmolality attributable to high concentrations of glucose have been shown to influence PTH secretion by dispersed parathyroid cells in vitro (45).

X-ApEn provides a measure of the synchrony of change between 2 variables over time (35). As noted previously for ApEn, X-ApEn for \(\text{iCa}^{2+}\) and PTH were lower in patients receiving calcium-containing TPN than in healthy volunteers, indicating greater synchrony between these 2 variables when calcium was present in nutrient solutions. The reduction in X-ApEn for \(\text{iCa}^{2+}\) and PTH in patients receiving calcium-containing TPN is again probably related to the infusion of relatively large amounts of calcium intravenously over a few hours, thereby inducing temporally predictable increases in \(\text{iCa}^{2+}\) concentrations and corresponding reductions in serum PTH concentrations. After calcium was removed from the TPN solutions, X-ApEn for \(\text{iCa}^{2+}\) and PTH increased, and values did not differ significantly from those observed in healthy volunteers. Thus, calcium restriction decreased the synchrony of change between \(\text{iCa}^{2+}\) and PTH throughout the day and restored a pattern of change more similar to that seen in the healthy volunteers. Of interest in this regard, greater moment-to-moment irregularity of heartbeat is physiologically normative compared with the inappropriately regularized interpeak interval observed in sudden infant death syndrome (46).

It remains to be determined whether disturbances in the intrinsic secretory behavior of the parathyroid glands modify bone mass and skeletal remodeling in long-term TPN recipients. An irregular pattern of PTH release from the parathyroid glands may be important, however, in modulating expression of the PTH–PTH-related peptide (PTHrP) receptor in target tissues, including bone. Increases in the regularity of PTH secretion together with moderate but persistent elevations in serum PTH concentrations may lower PTH–PTHrP receptor expression and diminish the response to PTH at the level of the osteoblast; such changes could contribute to reductions in bone formation and turnover. In this regard, differences between sustained elevations in serum PTH concentrations in primary hyperparathyroidism and fluctuating hormone concentrations during the intermittent

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**FIGURE 4.** Mean (±1 SD) approximate entropy (ApEn) for blood ionized calcium (□) and serum parathyroid hormone (■) concentrations in 10 volunteers with normal renal and parathyroid gland function and in 6 patients receiving long-term total parenteral nutrition (TPN) with and without calcium. * Significantly different from healthy volunteers, \(P < 0.05\).

**FIGURE 5.** Mean (±1 SD) cross approximate entropy (X-ApEn) for blood ionized calcium and serum parathyroid hormone in 10 volunteers with normal renal and parathyroid gland function and in 6 patients receiving long-term total parenteral nutrition (TPN) with and without calcium. * Significantly different from healthy volunteers, \(P < 0.05\).
administration of exogenous PTH are thought to account for disparate effects on bone mass. Thus, bone mass is often reduced in patients with primary hyperparathyroidism, particularly in cortical bone in the appendicular skeleton, whereas the amount of cancellous bone increases after the intermittent administration of synthetic PTH to patients with osteoporosis (47–51).

The results of the current investigation show that infusions of calcium-containing TPN solutions strikingly eliminate the normal nocturnal rise in serum PTH concentrations. Although the physiologic importance of nyctohemeral changes in PTH secretion remains uncertain, the diurnal variation in serum PTH concentrations is lost in patients with primary hyperparathyroidism, many of whom have reductions in bone mass. In contrast, greater nocturnal increases in serum PTH concentrations were implicated as a factor that contributes to the increase in bone mass during treatment with the bis-phosphonate alendronate (14). Additional work is required, however, to determine whether altering the schedule of TPN infusions affects total body calcium balance in patients receiving long-term TPN.

In summary, parathyroid gland function was abnormal and there was evidence of mild secondary hyperparathyroidism in the patients who received long-term TPN. Nocturnal infusions of calcium-containing TPN solutions disrupted the normal diurnal variations in iCa\(^{2+}\) and serum PTH concentrations and eliminated the characteristic nocturnal rise in serum PTH concentrations. Both the amplitude of change throughout the day and the orderliness of characteristic nocturnal rise in serum PTH concentrations were greater in patients given calcium-containing TPN solutions than in the healthy volunteers, who had normal renal and parathyroid gland function. The effect of these disturbances on bone and mineral metabolism remains to be determined, but the results suggest one mechanism to account for reductions in osteoblastic activity and bone formation in patients receiving long-term TPN.

REFERENCES