A longitudinal study of calcium regulation in a nonhuman primate model of parenteral nutrition¹⁻³

Edward W Lipkin

ABSTRACT The question of whether parenteral nutrition adversely affects calcium regulation remains unclear. Human studies of this question have been confounded by uncontrolled variables including the degree to which food is ingested orally; gut absorption; underlying disease; medication adversely affecting bone, including steroids; and aluminum contamination of parenteral nutrients. The present study was undertaken to examine whether parenteral nutrition adversely affects calcium regulation in a nonhuman primate model that allows for control of underlying clinical variables and mobility. With use of this model, it was possible to show weight maintenance and positive nitrogen and calcium balances with parenteral nutrition. There was no demonstrable effect of the animals' wearing a jacket and tether system or of catheterization on calcium regulation. Calciuria in response to parenteral nutrition was elevated initially but diminished by 2 wk of therapy. The calciuria observed resulted from an increased urine-filtered calcium load. Calcium balance with parenteral nutrition was preserved by a diminished fraction of calcium filtered by the kidney being excreted in the urine. The present study suggests that negative calcium balance produced by parenteral nutrition may result from abnormal renal tubular function or disruption of normal parathyroid hormone regulation.

INTRODUCTION Parenteral nutrition, which can support weight and lean body mass in individuals with gastrointestinal disease, has been associated with negative calcium balance, hypercalciuria, and metabolic bone disease in selected individuals (1–5). Limited longitudinal studies have suggested that these events may be affected by the duration of therapy (1, 6–8). Most data in humans, however, have been confounded by variable degrees of rehabilitation, underlying diseases, medications, and aluminum exposure. This laboratory has been involved in developing a nonhuman primate model of parenteral nutrition. A suitable animal model offers the advantage of not being confounded by uncontrolled variables, and the nonhuman primate is an ideal model for metabolic bone disease in humans because it mimics the human physiology of bone (9). The current study was initiated in our nonhuman primate model of parenteral nutrition to specifically ask the question of whether the negative calcium balance associated with parenteral nutrition changes with time. The present study was also designed to characterize the time course of changes in blood hormones that are associated with calcium regulation.

MATERIALS AND METHODS

Study design

The study was implemented as a two-by-two design in which one variable of the design was the effect of catheterization and wearing a jacket and tether system on calcium homeostasis and the other variable was the effect of parenteral versus oral administration of nutrients. The study thus depended on establishing four study groups, as follows: group 1, eight animals fed total parenteral nutrition (TPN) solution intravenously; group 2, six animals fed TPN solution orally; group 3, six animals fed an oral research diet; and group 4, seven animals fed an oral research diet and catheterized. Metabolic balance studies were initiated after a 2-wk stabilization period that allowed for dietary adaptation and recovery from invasive procedures. Each balance study lasted 6 d.

Animals

Twenty-seven healthy adult male nonhuman primates (Macaca fascicularis) were selected for investigation on the basis of stable weight histories, normal results from physical examination, and normal laboratory values for white blood cell count, hematocrit, serum electrolytes, blood urea nitrogen, creatinine, glucose, and bilirubin.

Central venous catheter

Single-lumen silastic catheters were surgically placed in animals under fenfluramine general anesthesia into the right or left central vein as previously described (10).
subclavian, femoral, or internal jugular vein as described previously (10). The catheters were tunneled subcutaneously to the midback, where they exited the skin. The intravenous line was protected with a jacket and tether system that allowed the animal unrestricted activity within the cage (10). Some animals had a catheter inserted under the same conditions that was exteriorized through the back, tied off, and then the end sutured beneath the skin. These animals were then placed in a jacket and tether system identical to that used for animals fed intravenously.

Nutrition

Eight animals were infused with TPN solution providing 5% amino acids at a rate paired to the rate of consumption of animals fed TPN solution orally. The additional components of the TPN solution are listed in Table 1. The TPN solution was delivered to provide 252–294 kJ · kg⁻¹ · d⁻¹ (≈60–70 kcal · kg⁻¹ · d⁻¹) through the single lumen catheter. The TPN solution contained methionine as 5.7% of the amino acid content (by wt) but no cysteine. Infusion rates were controlled with a Holter roller pump (Extraorporeal Medical Specialties, Inc, King of Prussia, PA). Infusion rates were confirmed daily by measuring the volume of solution infused over 1 h in addition to calculating infusion rates based on daily changes in solution bag weights. Animals receiving TPN were given one-quarter of an apple per day (40–50 g) as a source of fiber. The weight of apple consumed daily was recorded and an average daily consumption was calculated. Apples were estimated to contain 85% water by weight and 2.5 kJ (11), 15 mg dietary fiber (12), or 7.7 mg crude fiber (11) per g.

Two comparison groups of animals were pair fed a weighed quantity of a synthetic polymeric diet (ICN Biomedicals, Inc, Costa Mesa, CA). The polymeric diet contained 217 mg protein and 10.3 mg Ca/g. The protein source in the metabolic diet was casein and the fat content was 6.0% (by wt) provided as corn oil. Casein contains 2.6% (by wt) of its amino acid content as methionine and 0.4% (by wt) of its amino acid content as cysteine.

Animals receiving TPN solution orally were fed solution supplemented immediately before use with additional calcium as calcium gluconate and phosphorus as sodium phosphate with the intent of compensating for the reduced absorption of these nutrients when consumed orally (13–16). The solution was administered under gravity through a sipper and tube apparatus. The relative intakes of vitamin D were 9.1 fmol/kJ energy for the metabolic diet and 5.1 fmol/kJ energy for the TPN solutions. The ratio of calcium to phosphorus was maintained at > 0.30 for the TPN groups because a ratio > 0.23 is felt to be adequate for non-human primates (17).

Animal care

Animals resided in separate metabolic cages that allow for the collection of urine and stool samples during metabolic studies (10). Animals were housed and cared for by the Regional Primate Research Center at the University of Washington. The housing environment included a constant temperature and light cycle for all study animals. There was no sun exposure; light exposure was uniform fluorescent light. The protocol was approved by the Animal Care Review Committee. The water intake for each animal was measured daily. Animal weights were obtained immediately before the initiation of TPN at the time of surgical catheter placement and weekly thereafter. Weekly blood samples were obtained from animals surviving a 7-d TPN stabilization period by femoral venipuncture while animals were under ketamine anesthesia. Serum glucose, total bilirubin, albumin, and total protein were measured before and during weekly TPN support after a 7-d stabilization period. All metabolic studies were started 7 d after the introduction of the research diets. Urine and stool were collected separately over two 3-d intervals. Results for each week were the average of both collections. Mineral balance was measured for 3 successive weeks.

Chemical analyses

Serum total calcium was measured colorimetrically with an autoanalyzer (Paramax; American-Dade Chemistry Systems, Costa Mesa, CA) and ionized serum calcium with an ion-specific electrode (Radiometer, Copenhagen) (18). Calcium in stools and urine was measured by atomic-absorption spectroscopy (19). Stool was prepared for analysis of calcium by ashing, followed by acid extraction. The intraassay CV for calcium measurements was < 2%.

Total nitrogen was measured in urine by chemiluminescence (20). The intraassay CV for this measurement was 2%. Urinary hydroxyproline was measured spectrophotometrically after treatment of the urine with an ion-exchange resin and activated charcoal, acid hydrolysis, oxidation to pyrrole, and reaction with a chromophore (21). 25-Hydroxyvitamin D was measured by competitive protein binding (22), 1,25-dihydroxyvitamin D by competitive binding to chick thymus receptor (23), and intact
parathyroid hormone (PTH) by a double-site radioimmunoassay (24). Nitrogen intake was calculated as TPN nitrogen plus measured apple nitrogen over 72 h. Total nitrogen output was measured from 72-h urine and stool collections. Apple and stool nitrogen concentrations were measured by the Kjeldahl technique (25). Averages of two 72-h collection periods were used to determine nitrogen, mineral, and sodium losses. Oral intakes of nutrients were quantified from the weight of oral diet consumed and the composition of the diet.

**RESULTS**

Intakes of energy, nitrogen, calcium, phosphorus, and magnesium and urinary sodium and hydroxyproline are shown in Table 2. The four study groups were well matched for energy intake. Nitrogen intake of the group fed TPN solution orally was less. The four study groups were well matched for energy intake. The group fed TPN solution orally had significantly diminished nitrogen balance compared with both groups fed the oral research diet (those with and without catheters) and a significantly different pattern of weight change compared with all other study groups (Table 3). None of the changes in weight were significantly different from zero, however, and all mean plasma concentrations of insulin-like growth factor I were similar.

Mean intakes of calcium, phosphorus, and magnesium were less for both groups receiving TPN solution (intravenously or orally) than for both groups fed the oral research diet (Table 2). Additionally, mean nitrogen balances in the two groups fed TPN solution were significantly less in the two groups fed the oral research diet (Table 3). No significant differences in mean nitrogen balance were found between the groups who received TPN solution orally or intravenously. Mean plasma concentrations of 25-hydroxyvitamin D were also significantly less in both groups fed TPN solution than in both groups fed the oral research diet; there were no significant differences in plasma concentrations of 1,25-dihydroxyvitamin D or PTH (Table 3). The mean intake of vitamin D (ergocalciferol) was 11.5 nmol/d in the groups fed the oral research diet and 6.84 nmol/d in the groups fed TPN solution.

The only treatment group in which a significant trend for change in calcium metabolism over different collection periods could be identified was the group supported by intravenous TPN (Tables 4 and 5). Calcium decreased and net calcium balance improved from the first to the second collection period and was maintained from the second to the third collection period in this group. Mean net calcium and phosphorus absorption was negative throughout the study in this group as well (Table 6).

Calcium absorption was 74 ± 13% of intake for the group fed TPN solution orally and 40 ± 10% for the groups fed the oral research diet; phosphorus absorption was 58 ± 7% of intake and 14 ± 13% of intake for these same groups, respectively. The net retention of calcium was 37 ± 19% for the group fed TPN solution orally and 3.7 ± 1.7% for the groups fed the oral research diet; the net retention of phosphorus was 27 ± 11% and 8.5 ± 1.5% in these same groups, respectively. The changes in calcium homeostasis were not paralleled by changes in phosphorus (Table 7). There were no statistically significant changes in urinary phosphorus or in phosphorus balances over time or between different groups.

Mean plasma PTH was more than twofold higher in the groups fed TPN solution than in the groups fed the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter. Values in the same row with different superscript letters are significantly different, P < 0.05 (one-way ANOVA and Tukey’s honestly significantly different post hoc test).

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### Table 2

| Nutrient Intakes, Urinary Sodium, and Urinary Hydroxyproline in Different Study Cohorts[^a] |
|---------------------------------|-------|-------|-------|-------|
|                                | Group 1 (n = 8) | Group 2 (n = 6) | Group 3 (n = 6) | Group 4 (n = 7) |
| Energy intake (kJ · kg⁻¹ · d⁻¹) | 282 ± 41  | 269 ± 67  | 257 ± 67  | 278 ± 31  |
| Nitrogen intake (mmol · kg⁻¹ · d⁻¹) | 34.3 ± 5.0a  | 32.1 ± 7.9a  | 40.7 ± 10.7ab  | 44.3 ± 5.0b  |
| Ca intake (mmol/d)              | 0.83 ± 0.23b  | 1.96 ± 0.80b  | 16.3 ± 6.0b  | 19.5 ± 4.5b  |
| P intake (mmol/d)               | 2.33 ± 0.70b  | 3.49 ± 1.59b  | 11.1 ± 1.0b  | 12.2 ± 1.6b  |
| Mg intake (mmol/d)              | 1.37 ± 0.34a  | 1.13 ± 0.47a  | 3.11 ± 0.28b  | 3.41 ± 0.44b  |
| Urinary Na (mmol/d)             | 6.83 ± 3.91a  | 5.48 ± 2.09a  | 9.70 ± 2.39ab | 12.6 ± 2.6a  |
| Urinary hydroxyproline (μmol/nmol creatinine) | 66.9 ± 18.5 | 54.3 ± 17.3 | 60.2 ± 29.8 | 66.2 ± 24.6 |

[^a]: X ± SD. Groups were as follows: 1, received total parenteral nutrition (TPN) intravenously; 2, received TPN orally; 3, received the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter. Values in the same row with different superscript letters are significantly different, P < 0.05 (one-way ANOVA and Tukey’s honestly significantly different post hoc test).
diet (9 ± 2.5 compared with 11.7 ± 7.1 pmol/L; Table 8), but none of the differences were significant. In the group receiving TPN solution intravenously, a significant correlation was observed between calcitriol and creatinine clearance (r = 0.90, P = 0.002, n = 8; Figure 1), between calcitriol and the calcium filtration fraction (r = 0.92, P = 0.002, n = 8; Figure 2), and between calcitriol and plasma PTH concentrations (r = –0.82, P = 0.013, n = 8; Figure 3). There was a negative correlation between creatinine clearance and plasma PTH concentrations during TPN (r = –0.78, P = 0.024, n = 8), and between the calcium filtration fraction and plasma PTH concentrations (r = –0.78, P = 0.03, n = 8). Changes in calcium balance correlated with changes in PTH concentrations (r = –0.68, P = 0.09, n = 7). One follow-up PTH measurement was lost and not available for analysis of the complete data (n = 8) for change in PTH. A similar pattern was not observed in any of the other groups.

The degree of calcitriol observed in the group receiving TPN solution intravenously was less than in either of the groups fed the oral research diet (Table 9). This diminished calcitriol in the TPN-supported group was proportional to the diminished calcium intake of this group. The calcitriol observed in the group fed TPN solution orally was less than in the group with a blind catheter fed the oral research diet. The correlation between calcitriol and calcium intake was 0.73 (P < 0.00001, n = 27) for all study animals. A significant correlation was also apparent between calcitriol and urinary sodium (r = 0.68, P = 0.0001, n = 26; Figure 4) for all study animals. No significant correlation was found between calcitriol with either energy intake per kilogram of body weight or nitrogen intake per kilogram of body weight.

No significant differences in serum calcium were observed. The mean filtered urinary calcium concentration during the first collection period increased in the group fed TPN solution intravenously compared with that in both the group fed TPN solution orally and the group with a blind catheter fed the oral research diet (Table 5), but this was not significant (F = 1.85, P = 0.17, ANOVA). The mean net filtered urinary calcium for the TPN group overall was elevated (Table 9) compared with the other groups, but again this was not significant (F = 2.48, P = 0.09, ANOVA). Paradoxically, the calcium filtration fraction was significantly less in the group fed TPN solution intravenously than in the groups fed the oral research diet. The calcium filtration fraction was also significantly less in the group fed TPN solution orally than in the group with a blind catheter fed the oral research diet.

**DISCUSSION**

Studies have documented depletion of bone mineral in patients dependent on long-term parenteral nutrition (1, 2). Much of the older data, however, are confounded by the presence of either unknown aluminum burdens or overt aluminum toxici-
ty (26). These earlier studies were furthermore confounded by additional uncontrolled variables, including underlying disease, medications, and lack of information about mobility. Many patients dependent on parenteral nutrition may have metabolic bone disease at the initiation of therapy (3, 27–29). Disorders of calcium metabolism are frequent with parenteral nutrition therapy but can occur with or without metabolic bone disease. In two more studies (30, 31), three of six and one of five TPN-dependent patients, respectively, were markedly hypercalciuric. The authors of the first study were also unable to find marked hypercalciuria in any long-term parenteral nutrition patients with documented bone pathology, and suggested that there may be an adaptive response to hypercalciuria over time, with hypercalciuria being more characteristic of the short-term patient but not so marked in the long-term patient. Subsequent studies in both rats (32) and humans (33) documented a time-dependent decrease in urinary calcium loss in TPN-dependent subjects with massive bowel resections. A variety of factors have been established that determine the magnitude of calcium loss in the urine with parenteral nutrition infusion (30, 34, 35). These factors include renal function; protein, glucose, and sodium load; phosphate and base intake; and the infused dose of calcium. A subsequent reevaluation of the calciuria issue in human TPN studies suggested that in the absence of aluminum intoxication, intravenous calcium administration with TPN was not handled differently from an oral calcium bolus (36).

The ability of current parenteral therapies to promote optimal bone and calcium health is an object of investigation. Older studies are confounded by the use of therapies heavily contaminated with aluminum, by the use of therapies containing unphysiologic quantities of vitamin D, or by being only cross-sectional in design and not including long-term follow-up. Longitudinal data are limited. To resolve the difficulties of interpreting the human clinical studies of the effects of TPN on calcium regulation, we designed a nonhuman primate model of parenteral nutrition that allows for mobility of the experimental animal with minimal restraint and in addition to control for the effects of underlying disease, catheterization, and any restraint imposed by maintaining venous access. In the present study, the system also allowed for the maintenance of positive nitrogen balance and weight stability for periods of £6 wk. It was not possible to show a negative calcium balance with the use of TPN in the absence of

TABLE 5
Effects of treatment on longitudinal measures of calcium

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 6)</th>
<th>Group 4 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st SICa (mmol/L)</td>
<td>1.24 ± 0.05</td>
<td>1.24 ± 0.04</td>
<td>1.21 ± 0.04</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>2nd SICa (mmol/L)</td>
<td>1.24 ± 0.04</td>
<td>1.23 ± 0.02</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>3rd SICa (mmol/L)</td>
<td>1.24 ± 0.04</td>
<td>1.22 ± 0.02</td>
<td>1.22 ± 0.02</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>1st FCa (μmol/min)</td>
<td>17.8 ± 6.2</td>
<td>11.1 ± 3.9</td>
<td>11.3 ± 3.2</td>
<td>11.0 ± 2.2</td>
</tr>
<tr>
<td>2nd FCa (μmol/min)</td>
<td>16.8 ± 5.9</td>
<td>11.8 ± 5.4</td>
<td>11.8 ± 5.4</td>
<td>10.6 ± 2.6</td>
</tr>
<tr>
<td>3rd FCa (μmol/min)</td>
<td>14.1 ± 6.2</td>
<td>10.3 ± 4.1</td>
<td>11.0 ± 3.4</td>
<td>11.1 ± 2.7</td>
</tr>
<tr>
<td>1st FFCa</td>
<td>0.024 ± 0.021</td>
<td>0.047 ± 0.015</td>
<td>0.047 ± 0.015</td>
<td>0.138 ± 0.098</td>
</tr>
<tr>
<td>2nd FFCa</td>
<td>0.017 ± 0.023</td>
<td>0.044 ± 0.017</td>
<td>0.101 ± 0.038</td>
<td>0.143 ± 0.090</td>
</tr>
<tr>
<td>3rd FFCa</td>
<td>0.013 ± 0.012</td>
<td>0.053 ± 0.010</td>
<td>0.102 ± 0.027</td>
<td>0.134 ± 0.078</td>
</tr>
</tbody>
</table>

$\bar{x} \pm \text{SD.}$ Groups were as follows: 1, received total parenteral nutrition (TPN) intravenously; 2, received TPN orally; 3, received the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter. SICa, serum-ionized calcium; FCa, urine-filtered calcium; FFCa, calcium urine filtration fraction.

Significant main effect of time, $P < 0.032.$

TABLE 6
Effects of treatment on longitudinal measures of calcium and phosphorus absorption

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 6)</th>
<th>Group 4 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Net Ca absorption (mmol/d)</td>
<td>$-0.05 \pm 0.12$</td>
<td>$1.52 \pm 0.77$</td>
<td>$5.50 \pm 2.69$</td>
<td>$7.53 \pm 3.1$</td>
</tr>
<tr>
<td>2nd Net Ca absorption (mmol/d)</td>
<td>$-0.09 \pm 0.24$</td>
<td>$1.44 \pm 0.80$</td>
<td>$7.47 \pm 2.53$</td>
<td>$7.93 \pm 4.2$</td>
</tr>
<tr>
<td>3rd Net Ca absorption (mmol/d)</td>
<td>$-0.10 \pm 0.09$</td>
<td>$1.54 \pm 0.73$</td>
<td>$7.97 \pm 2.90$</td>
<td>$23.5 \pm 12.4$</td>
</tr>
<tr>
<td>1st Net P absorption (mmol/d)</td>
<td>$-0.52 \pm 0.59$</td>
<td>$0.73 \pm 1.99$</td>
<td>$2.18 \pm 1.11$</td>
<td>$1.42 \pm 1.74$</td>
</tr>
<tr>
<td>2nd Net P absorption (mmol/d)</td>
<td>$-0.27 \pm 0.34$</td>
<td>$2.01 \pm 1.12$</td>
<td>$2.65 \pm 0.88$</td>
<td>$1.13 \pm 3.16$</td>
</tr>
<tr>
<td>3rd Net P absorption (mmol/d)</td>
<td>$0.02 \pm 0.86$</td>
<td>$1.98 \pm 0.91$</td>
<td>$2.86 \pm 1.60$</td>
<td>$1.11 \pm 2.34$</td>
</tr>
</tbody>
</table>

$\bar{x} \pm \text{SD.}$ Groups were as follows: 1, received total parenteral nutrition (TPN) intravenously; 2, received TPN orally; 3, received the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter.
The increased calciuria observed in the groups fed the oral research diet can be attributed to increased solute load, including both calcium and sodium. The intravenous administration of TPN resulted in a more substantial calciuria over the short term compared with long-term administration. This effect was not characteristic of orally ingested TPN. The differing effects of oral and intravenous TPN suggest that the differences cannot be attributed to volume or sodium alone. The solutions were isovolemic and isonatremic and urine output was equivalent for all study groups. The effect of TPN on calciuria was correlated with the glomerular filtration rate, the urine filtration fraction for calcium, and the plasma PTH concentration. The initial period of intravenous TPN support was accompanied by a relative hyperfiltration of calcium, which diminished with time. The mechanism or mechanisms of calcium hyperfiltration were not elucidated but the hyperfiltration was consistent with increased renal blood flow. The current data suggest that adaptations occur with time in TPN therapy, which result in calcium conservation, including a diminished filtered calcium load and increased PTH concentrations in blood.

Urinary hydroxyproline measures in the current study did not suggest that increased bone resorption accounts for the calciuria associated with TPN. Although there are newer measures of bone resorption, including urinary N-telopeptide and serum type I collagen (37), urinary hydroxyproline is a well-established measure of bone resorption that is suited for large numbers of samples. In older studies, urinary hydroxyproline was useful in differentiating states of increased bone resorption such as postmenopausal osteoporosis (38–40) and Paget disease (40). A direct renal tubular effect leading to conservation of calcium is suggested in the setting of TPN because these changes occur concomitantly with increased urine-filtered calcium but a diminished calcium filtration fraction. Alternatively, the current data may be explained by a transient suppression of bone formation with the initiation of TPN, which would lead to transient hypercalciuria and diminished calcium balance. With a subsequent increase in bone formation, calciuria and calcium balance would improve. The current studies did not assess longitudinally direct changes in bone formation, but such an effect would still not negate the current observations of the effects of TPN on renal calcium handling. The current study also suggests that phosphate handling by the kidney is a poor indicator of renal tubular function in the setting of TPN. The changes in urinary phosphorus and phosphorus balance did not parallel the changes in calcium. Additionally, urinary phosphorus was only a weak correlate of PTH ($r = -0.44$, $P = 0.28$, $n = 8$) during TPN infusion.

The current data can be criticized because a TPN solution that was supplemented with additional calcium and phosphorus was used for oral feeding. Human data suggest, however, that the fractional absorption of calcium is $\approx 0.45$ (13, 14) and calcium kinetic data in nonhuman primates confirm that calcium handling by this species is equivalent to that in humans. Some adjustment for diminished absorption of bone mineral by the oral route appears reasonable. Data on the absorption of oral calcium and phosphorus from TPN solutions are not available. Human data suggest that changes in the macronutrient composition of an oral diet do not significantly change calcium absorption (14). Studies using glucose or glucose polymer, however, suggest that as single nutrients, these compounds increase calcium absorption to $\approx 50\%$ (13). Because of the large dextrose concentration of TPN solution, 50% calcium absorption was assumed when we designed the TPN solution used for oral feeding. The 95% CI for estimated calcium absorption from the TPN solution on the basis of underlying disease in the current nonhuman primate model. At higher rates of intravenous nutrition support (343 kJ·kg$^{-1}$·d$^{-1}$, or 82 kcal·kg$^{-1}$·d$^{-1}$, and 46 mmol N·kg$^{-1}$·d$^{-1}$); however, it has been possible to show negative calcium balance in this nonhuman model of parenteral nutrition (KE Friday, EW Lipkin, unpublished observations, 1996). There was also no specific effect of catheterization per se on calcium in the current study.

The increased calciuria observed in the groups fed the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter.

### TABLE 7
Effect of treatment on longitudinal measures of urinary phosphorus and phosphorus balance

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 6)</th>
<th>Group 4 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Urinary P (mmol/d)</td>
<td>1.00 ± 0.47</td>
<td>0.98 ± 0.60</td>
<td>1.08 ± 0.59</td>
<td>1.14 ± 0.75</td>
</tr>
<tr>
<td>2nd Urinary P (mmol/d)</td>
<td>0.95 ± 0.77</td>
<td>0.70 ± 0.44</td>
<td>0.79 ± 0.43</td>
<td>0.87 ± 0.56</td>
</tr>
<tr>
<td>3rd Urinary P (mmol/d)</td>
<td>1.22 ± 1.12</td>
<td>0.82 ± 0.60</td>
<td>0.76 ± 0.49</td>
<td>0.50 ± 0.33</td>
</tr>
<tr>
<td>1st Net P (mmol/d)</td>
<td>0.85 ± 0.48</td>
<td>0.64 ± 1.16</td>
<td>0.59 ± 1.80</td>
<td>0.13 ± 1.59</td>
</tr>
<tr>
<td>2nd Net P (mmol/d)</td>
<td>0.96 ± 0.45</td>
<td>1.18 ± 0.67</td>
<td>1.55 ± 1.49</td>
<td>0.20 ± 2.72</td>
</tr>
<tr>
<td>3rd Net P (mmol/d)</td>
<td>0.51 ± 1.15</td>
<td>0.72 ± 1.24</td>
<td>1.73 ± 1.16</td>
<td>2.29 ± 4.13</td>
</tr>
</tbody>
</table>

$^1 \bar{x} \pm SD$. Groups were as follows: 1, received total parenteral nutrition (TPN) intravenously; 2, received TPN orally; 3, received the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter.
of balance data from the current study was within the range of values reported in the literature for glucose-enhanced calcium absorption. The observed range of calcium absorbed from the oral TPN solution (0.71–2.53 mmol/d) was a reasonable approximation of the amount of calcium infused in the intravenous TPN group (0.62–1.28 mmol/d). Similarly, the observed range of phosphorus absorbed from the oral TPN solution (1.13–3.68 mmol/d) was a reasonable approximation of the amount of phosphorus infused in the intravenous TPN group (1.70–3.55 mmol/d). The negative estimated net calcium absorption in the TPN group is consistent with a small component of gastrointestinal calcium loss. The 95% CI for estimated phosphorus absorption from the current study was also within the range of supplementation used for the oral TPN group.

In the current study, a chemically defined polymeric diet promoted a more positive nitrogen balance than did TPN administered either orally or intravenously. The mechanism or mechanisms promoting this effect are not elucidated in the current study but several are possible. The animals’ body composition was not determined, but it is possible that TPN is associated with gut atrophy, which would result in less favorable nitrogen balance. It is also possible that the amino acid mixture of the TPN solution was not optimal for nonhuman primates or that differences in the intake of other nutrients such as fat (6% with the polymeric oral diet compared with 1.7% with TPN) were responsible for more efficient nitrogen utilization in the groups fed the polymeric research diet. Sulfur-containing amino acids (methionine and cysteine), which have a striking effect on calciuria, were 2.9% (by wt) of the amino acid content of the polymeric research diet and 5.7% (by wt) of the amino acid content of the TPN solution. These dietary differences between the TPN solution and the polymeric research diet may confound the current observations regarding calcium regulation with TPN.

In contrast with previous human studies, however, the current nonhuman primate study of the effects of TPN on calcium handling did not document any specific deleterious effects. The implications of the current study are that if TPN contributes directly to the osteopenia observed with long-term TPN, this may in selected individuals be due to impairment of the normal regulation of PTH and impaired renal tubular handling of calci-

### Table 9
Summary of renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine volume (mL/d)</th>
<th>Urinary creatinine (mmol/d)</th>
<th>Endogenous creatinine clearance (mL/min)</th>
<th>Urinary Ca (mmol/d)</th>
<th>Urine-filtered Ca (µmol/min)</th>
<th>Ca filtration fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>416 ± 225</td>
<td>1.53 ± 0.30</td>
<td>12.9 ± 4.2</td>
<td>0.37 ± 0.32</td>
<td>15.7 ± 5.2</td>
<td>1.43 ± 0.86</td>
</tr>
<tr>
<td>Group 2</td>
<td>385 ± 201</td>
<td>1.17 ± 0.36</td>
<td>9.6 ± 3.6</td>
<td>0.88 ± 0.44</td>
<td>11.8 ± 4.7</td>
<td>5.02 ± 0.92</td>
</tr>
<tr>
<td>Group 3</td>
<td>242 ± 141</td>
<td>1.26 ± 0.37</td>
<td>9.3 ± 2.4</td>
<td>1.62 ± 0.36</td>
<td>11.4 ± 3.0</td>
<td>10.4 ± 3.2</td>
</tr>
<tr>
<td>Group 4</td>
<td>304 ± 118</td>
<td>1.28 ± 0.20</td>
<td>8.8 ± 1.7</td>
<td>2.19 ± 1.40</td>
<td>10.6 ± 2.1</td>
<td>14.6 ± 9.3</td>
</tr>
</tbody>
</table>

* ± SD. Groups were as follows: 1, received total parenteral nutrition (TPN) intravenously; 2, received TPN orally; 3, received the oral research diet and had no catheter; and 4, received the oral research diet and had a catheter. Values in the same row with different superscript letters are significantly different, P < 0.05 (one-way ANOVA and Tukey’s honestly significantly different post hoc test).

**Figure 1.** Correlation between urinary calcium and creatinine clearance for animals maintained on total parenteral nutrition intravenously. $r = 0.90, P = 0.002, n = 8.$

**Figure 2.** Correlation between urinary calcium and the calcium filtration fraction for animals maintained on total parenteral nutrition intravenously. $r = 0.92, P = 0.002, n = 8.$
um. A central role for PTH in preserving spinal bone content was suggested by Verhage et al (41). Interestingly, aluminum is a former contaminant of intravenous therapies that has the potential for impairing both normal PTH regulation and renal tubular handling of calcium. Both mechanisms may be contributing factors to the metabolic bone disease observed in long-term parenteral therapy with solutions contaminated with aluminum (42, 43).

The current results in an animal model substantiate our findings (6) and those of others (41) in humans that present-day parenteral nutrition formulations do not have deleterious effects on bone health in a heterogeneous population with established bone pathology. Findings from the current studies are also consistent with our previous suggestion that bone mineralization in a significant number of parenteral nutrition patients can be either improved or stabilized by parenteral therapy. The study by Staun et al (7) also documented stability or improvement in lumbar spine bone mineral content in some individuals despite overall deterioration in their study group of 4%/y. The study by Lawson et al (44) in young growing rats also suggests that the current findings do not apply to an animal model in a rapid growth phase where calcium availability may be a rate-limiting factor in bone growth.

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REFERENCES


![Figure 3](image-url)  
**FIGURE 3.** Correlation between urinary calcium and plasma parathyroid hormone (PTH) concentration for animals maintained on total parenteral nutrition intravenously. $r = -0.82, P = 0.013, n = 8.$

![Figure 4](image-url)  
**FIGURE 4.** Correlation between urinary calcium and urinary sodium for all animals in the study. $r = 0.68, P = 0.0001, n = 26.$


