Effect of phosphorus on endogenous calcium losses during total parenteral nutrition1-3

Richard J Wood, PhD; Michael D Sitrin, MD; and Irwin H Rosenberg, MD

ABSTRACT Intravenous phosphorus can reduce urinary calcium losses in patients receiving total parenteral nutrition (TPN). We investigated the effect of intravenous P on urinary and fecal Ca loss in intravenously fed normal and thyroparathyroidectomized (TPTX) rats to assess the role of parathyroid hormone (PTH) and endogenous fecal Ca losses. Doubling the intravenous P load during TPN decreased urinary Ca losses by 54% (0.299 vs 0.137 mmol/d) in intact rats and by 43% (0.514 vs 0.294 mmol/d) in TPTX rats. Increased P load in normal rats had no effect on urinary cyclic AMP excretion, serum Ca, serum P, serum 1,25-dihydroxycholecalciferol, or endogenous fecal Ca losses. These observations suggest that the hypocalciuric effect of P during TPN is independent of PTH and is not caused by a repartitioning of obligatory Ca losses from the renal to the intestinal route. Am J Clin Nutr 1988;48:632-6.

KEY WORDS Total parenteral nutrition, hypercalciuria, rat, phosphorus, calcium metabolism, endogenous fecal calcium

Introduction

Hypercalciuria is common in patients receiving total parenteral nutrition (TPN) (1, 2). Moreover, patients on long-term home TPN may develop metabolic bone disease (3). Excessive urinary calcium losses could exacerbate the rate of bone loss in these patients, thereby increasing their risk of developing osteopenia. The etiology of TPN-induced hypercalciuria is uncertain and may be multifactorial. However, the degree of urinary Ca loss can be modified by the nutritional composition of the TPN prescription (1, 4, 5). For example, we showed previously in both TPN patients and in rats receiving TPN that amino acids increase urinary Ca losses (1, 5). In contrast increased intravenous phosphorus administration in TPN patients has a hypocalciuric effect (4).

The hypocalciuric action of P has been known for > 50 y (6). Studies by various investigators suggested that P could decrease urinary Ca losses although there is some question as to whether this effect is via a parathyroid hormone(PTH)-dependent (7) or a PTH hormone-independent (8) renal mechanism. In addition, other studies suggested that P can decrease intestinal Ca absorption (9) and is associated with increased intestinal Ca secretion (10).

Either a renal reabsorptive or intestinal mechanism could reduce urinary Ca loss. However, which mechanism of action causes the hypocalciuric effect of P during TPN is important from a nutritional and clinical point of view because if P administration causes increased PTH secretion, then an untoward augmentation of bone loss or clinically significant hypercalciuria and soft tissue calcification could occur. On the other hand, an increase of endogenous fecal Ca loss during TPN would lessen the net effect of any decrease in urinary Ca excretion on Ca balance.

We assessed the effect of P on urinary Ca excretion and endogenous fecal Ca losses in a hypercalciuric TPN model (5) in normal and thyroparathyroidectomized (TPTX) rats.

Methods

Surgical procedure

Male Sprague Dawley rats (Holzman, Madison, WI) weighing ~200 g were surgically prepared to receive continuous intravenous nutrition as described previously (5). Briefly, ani-
TABLE I
Composition of infusate for TPN rats

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids (g/L)*</td>
<td>42.5</td>
</tr>
<tr>
<td>Dextrose (g/L)†</td>
<td>30</td>
</tr>
<tr>
<td>Minerals (mmol/L)‡</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>40</td>
</tr>
<tr>
<td>Potassium</td>
<td>24</td>
</tr>
<tr>
<td>Chloride</td>
<td>24</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7</td>
</tr>
<tr>
<td>Calcium</td>
<td>19</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>10 or 20</td>
</tr>
<tr>
<td>Trace elements (µmol/L)§</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>15</td>
</tr>
<tr>
<td>Copper</td>
<td>25</td>
</tr>
<tr>
<td>Zinc</td>
<td>98</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamins (µmol/L)¶</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>511</td>
</tr>
<tr>
<td>Retinol</td>
<td>2</td>
</tr>
<tr>
<td>Ergocalciferol</td>
<td>0.01</td>
</tr>
<tr>
<td>Thiamin HCl</td>
<td>27</td>
</tr>
<tr>
<td>Riboflavin phosphate</td>
<td>4</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>13</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>147</td>
</tr>
<tr>
<td>Dexpenthenol</td>
<td>22</td>
</tr>
<tr>
<td>All-rac-α-tocopherol acetate</td>
<td>2</td>
</tr>
</tbody>
</table>

* Travosal®, Travenol Laboratories, Inc, Deerfield, IL.
† Travenal Laboratories, Inc, Deerfield, IL.
‡ To keep the infusate sodium level constant, 10 mmol sodium acetate/L was eliminated from the infusate when the higher dose of P (given as sodium glycerophosphate) was used. Mg was supplied as magnesium sulfate and Ca was supplied as calcium gluconate.
§ IMS Ltd, So El Monte, CA.
¶ MVI Concentrate®, USV Laboratories, Tuckahoe, NY.

mals were anesthetized with ether and a small incision was made in the neck to insert a polyethylene catheter with a 3-cm silastic tip into the jugular vein. The catheter was secured to the vein by a suture and tunneled subcutaneously to the back of the neck where it exited. The catheter was secured to the animal by a stainless steel button and then connected to a swivel device secured to the top of the metabolic cage. This procedure allowed the animal to move freely about the cage while it received a continuous infusion of nutrients to supply its nutritional needs. All animals used in these experiments were cared for in accordance with the guidelines established by the National Research Council.

TPN procedure

All infusion solutions used in these experiments were prepared under sterile conditions, kept in sterile plastic TPN bags, and administered with a peristaltic pump at 2.5 mL/h in each experiment. After surgery all animals received 155 mmol NaCl/L at a rate of 2.5 mL/h overnight. The TPN solutions used in these experiments were patterned after those described by Tao et al (11) for the TPN rat and are shown in Table I. After the overnight saline infusion, animals were switched to the TPN solution which contained either 10 or 20 mmol/L P. The initial dosage of P was chosen on the basis of the formulation by Tao et al (11).

Studies in normal TPN rats

An experiment was conducted in normal TPN rats to determine whether 20 mmol P/L (n = 9) vs 10 mmol P/L (n = 10) would lower urinary Ca losses and whether changes in urinary Ca losses with increased P were associated with an increase in the PTH activity or an increase in endogenous fecal Ca losses. Urinary cyclic AMP (cAMP) excretion was used as an index of PTH activity (12). In addition we measured serum 1,25-dihydroxycholecalciferol because PTH is a trophic hormone for 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) production (13) and higher PTH levels would be expected to increase the serum levels of this vitamin D metabolite. Urine was collected in acid (2% vol/vol 6 mol HCl/L) on the third day of TPN, diluted to a standard volume, and stored frozen for subsequent analysis of Ca (14) and cAMP (15). Serum Ca (16) and P (17) were measured from the blood obtained at surgery and when the rats were killed. Serum 1,25-(OH)₂D₃ was measured in the final blood sample by a nonequilibrium competitive protein binding assay using a calf thymus receptor (18).

Endogenous fecal Ca (19) was determined in normal rats as follows: TPN rats were injected intravenously with 30 µCi (1.11 × 10⁶ Bq) radioactive ⁴⁵Ca and urine and feces were collected for 3 d at which time the animals were killed. At death their entire gastrointestinal tract was removed, cut open, and washed to recover any residual ⁴⁵Ca in the gut. Any feces produced during the 3 d and the washings were homogenized and an aliquot was treated by the dry-ash procedure at 550 °C overnight in a muffle furnace (Thermolyne 1300, Thermolyne Corp, Dubuque, IO). The samples were then dissolved in HCl, brought to a standard volume, and an aliquot was counted in a liquid scintillation counter (Tri-carb® 2000CA, Packard Instrument Co, Sterling, VA). These measurements were used to determine the total amount of ⁴⁵Ca radioactivity secreted into the intestine and not absorbed during the 3-d period. An equal fraction of each day’s standardized urine sample was pooled to obtain an estimate of the average specific activity of plasma over the 3-d collection period. Endogenous fecal Ca (EFC) excretion during the 3 d was calculated as total fecal ⁴⁵Ca divided by urinary specific activity.

Studies in thyroparathyroidectomized TPN rats

To determine whether PTH or thyroid hormone secretion mediates the lowering of urinary Ca losses by P during TPN, TPTX rats were given either 10 (n = 6) or 20 (n = 5) mmol P/L in their TPN infusate for 3 d. TPTX rats were thyroparathyroidectomized 7 d before TPN catheter implantation. Rats in which the surgery was successful were selected by serum Ca levels < 1.87 mmol/L after an overnight fast (20). Blood samples were collected as described above. Urine samples were collected on day 3 of TPN because preliminary studies showed that infusion of TPN solutions containing Ca restored serum Ca levels in TPTX rats to the normal range by day 3. Replacement thyroid hormones were not given during TPN.

Statistical analysis

The statistical significance (p < 0.05) of treatment effects was determined by the Student’s t test. All data in the text are presented as mean ± SEM.

Results

Effect of phosphorus in normal TPN rats

The effect of intravenous P on urinary Ca losses in animals receiving TPN is shown in Figure 1. Doubling the
concentration of P in the infusate from 10 to 20 mmol/L significantly decreased urinary Ca excretion in normal TPN animals by 54% (0.299 ± 0.06 vs 0.137 ± 0.30 mmol/d). Serum Ca, P, and 1,25-(OH)\(_2\)D and urinary cAMP excretion of normal animals receiving 10 or 20 mmol P/L are shown in Table 2. No significant differences were found for these variables as a result of doubling the rate of P administration during TPN infusion. Urinary P excretion increased significantly from 0.097 ± 0.023 to 0.572 ± 0.084 mmol/d after infusion of the higher P level. Endogenous fecal Ca losses during infusion of 10 mmol P/L (0.027 ± 0.01 mmol/d) were not different from losses during 20 mmol P/L infusion (0.030 ± 0.012 mmol/d).

**Effect of phosphorus in TPTX rats**

The effect of P on urinary Ca excretion in TPTX rats is shown in Figure 1. Urinary Ca excretion was significantly decreased by 43% (0.514 ± 0.065 vs 0.294 ± 0.022 mmol/d) in animals receiving the increased intravenous P load. No significant differences were found in serum Ca in TPTX rats receiving either 10 or 20 mmol P/L in their TPN solutions. The mean serum Ca level at the time of catheter placement was 1.60 ± 0.1 mmol/L in TPTX rats, which is significantly (p < 0.001) lower than the value for normal rats at the time of catheter placement, 2.45 ± 0.05 mmol/L. TPN administration with 19.2 mmol Ca/L normalized serum Ca levels in hypocalcemic TPTX animals by day 3 of TPN (1.60 vs 2.30 mmol/L; p < 0.001), whereas control animals receiving TPN remained normocalcemic (2.45 vs 2.54 mmol/L; p < 0.4).

**Discussion**

This study demonstrates that an increase in the P concentration of the TPN prescription can significantly decrease urinary Ca losses in the TPN rat without changing endogenous fecal Ca losses. We presume that this increase in Ca balance results in augmentation of skeletal Ca stores, but such augmentation was not determined directly in this acute experiment. However, because appreciable changes in the serum Ca-times-P product did not occur with the higher P load, we do not expect that the retained Ca ultimately precipitated out in soft tissues. The hypocalciuric effect of intravenous P in TPN rats is consistent with our previous observations in TPN patients that an increase from 22.6 to 42.0 mmol/d in the amount of P infused can cause a significant decrease in urinary Ca losses (4). In that study we also found no significant change in the serum Ca-times-P product.

Various studies (7, 21, 22) suggested that changes in PTH secretion could mediate P-induced decreases in urinary Ca excretion. Smith and Nordin (7) concluded from a study of eight osteoporotic patients that 48.4 mmol supplemental P induced a secondary hyperparathyroid state and thus reduced urinary Ca losses. Reiss et al (22) observed that 32.3 mmol oral P caused a transient postprandial decrease in serum ionized Ca and an increase in PTH levels in humans. Each of these studies found that intravenous Ca administration could prevent hypocalcemia and the subsequent increase in PTH activity. Recently, Silverberg et al (23) found that 64.6 mmol oral P/d caused an increase in PTH, urinary cAMP (an index of PTH activity), and serum 1,25-(OH)\(_2\)D in normal subjects, which rapidly returned to baseline levels despite continued P administration.

Our findings in this study of a hypocalciuric effect of P in TPTX rats is consistent with a PTH-independent action of P on renal Ca handling in animals receiving TPN. This notion is further strengthened by the observation that P was hypocalciuric even though it had no effect on either urinary cAMP or serum 1,25-(OH)\(_2\)D in normal TPN rats. These findings are in accord with those of Anderson and Draper (24), who found that increasing the P content of the diet of orally fed rats from 0.6 to 1.2% decreased urinary Ca in both intact and parathy-

**TABLE 2**

<table>
<thead>
<tr>
<th>Infusate P concentration</th>
<th>10 mmol/L</th>
<th>20 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca (mmol/L)</td>
<td>2.59 ± 0.02</td>
<td>2.50 ± 0.10</td>
</tr>
<tr>
<td>Serum P (mmol/L)</td>
<td>3.49 ± 0.13</td>
<td>3.55 ± 0.39</td>
</tr>
<tr>
<td>Serum 1,25-(OH)(_2)D (pmol/L)</td>
<td>56.2 ± 12.0</td>
<td>61.2 ± 21.2</td>
</tr>
<tr>
<td>Urinary cAMP (nmol/d)</td>
<td>131 ± 43</td>
<td>76 ± 31</td>
</tr>
</tbody>
</table>

* x ± SEM.
roidectomized (PTX) animals. Moreover, Lau et al (8) suggest a PTH-independent effect on Ca handling by short-term (3-h) P infusion with renal-Ca-clearance and micropuncture techniques in diuretic-treated, acutely PTX rats.

The effect of P on intestinal Ca handling has been a controversial subject (9, 10, 25). Edwards and Hodgkinson (9) found that P supplementation of hypercalcicuric subjects increased fecal Ca excretion but they did not attempt to determine whether this was due to decreased absorption of dietary Ca or to increased endogenous fecal Ca losses. Heaney and Recker (10) reported a statistical association in middle-aged women between increased dietary P intake and increased intestinal Ca secretion. These studies can be interpreted to suggest that a P-induced increase in intestinal Ca secretion could also be involved in the hypocalciuric effect of P by shunting a greater fraction of obligatory endogenous Ca losses through the intestinal rather than the renal route. If this occurs to a significant degree during TPN, the nutritional benefits of the hypocalciuric effect of phosphorus would be lessened.

Our study suggests that the role of the intestine in P-induced hypocalciuria is smaller than previously reported. The TPN rat receives all of its nutrients intravenously, thereby greatly diminishing the nutritional role of intestinal absorption. However, because these animals still have intact gastrointestinal tracts, the normal process of Ca secretion and absorption by the intestine continues. We observed, however, that only ∼0.025 mmol Ca/d is lost as obligatory fecal losses during TPN in the rat and, most importantly, this rate of loss was not affected by dietary P levels. This clearly demonstrates that in the TPN rat increased P intake did not reduce urinary Ca losses by merely shifting the route of a portion of obligatory endogenous Ca losses from the kidneys to the intestine. The lack of a differential effect of P in TPN rats is in accord with a recent report of Spencer et al (25) who found no effect of P intake on endogenous fecal Ca excretion in orally fed subjects. Interestingly, the degree of obligatory Ca loss via the intestinal route observed here during intravenous feeding is many times less than what typically occurs in orally fed rats absorbing levels of Ca similar to that given here intravenously (26, 27). Our study does not allow us to determine whether the low levels of endogenous fecal Ca losses are due to diminished secretion or to augmented absorption of secreted Ca. Nonetheless, from a nutritional point of view, this phenomenon could be important in maintaining Ca homeostasis during intravenous feeding.

Although P administration can be shown to trigger PTH release under certain circumstances, P can also clearly exert a hypocalciuric effect in the absence of changes in PTH. In light of our findings, this fact may have particular nutritional relevance during TPN, where infused Ca could presumably prevent the hypocalcemic effect of P and the subsequent stimulation of PTH release (7), thus achieving the nutritional benefits of a more positive Ca balance after a lower rate of urinary Ca loss due to P infusion. Moreover, these findings in TPN rats may have some relevance to TPN patients because we found that P administration to TPN patients for 3 d lowers urinary Ca losses without changing serum PTH or urinary cAMP excretion (28). However, additional clinical studies are needed to evaluate the safety and effectiveness of this and other (29) nutritional approaches to managing TPN-induced hypocalciuria over significantly longer periods of time.

We would like to thank Ms Evelyn O Gaston for her technical support throughout the course of these studies.

References


