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The Impact of Dietary Protein on Calcium Absorption and Kinetic Measures of Bone Turnover in Women

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Although high-protein diets induce hypercalciuria in humans, the source of the additional urinary calcium remains unclear. One hypothesis is that the high endogenous acid load of a high-protein diet is partially buffered by bone, leading to increased skeletal resorption and hypercalciuria. We used dual stable calcium isotopes to quantify the effect of a high-protein diet on calcium kinetics in women. The study consisted of 2 wk of a lead-in, well-balanced diet followed by 10 d of an experimental diet containing either moderate (1.0 g/kg) or high (2.1 g/kg) protein. Thirteen healthy women received both levels of protein in random order. Intestinal calcium absorption increased during the high-protein diet in comparison with the moderate (26.2 ± 1.9% *vs.* 18.5 ± 1.6%, $P < 0.0001$, mean ± SEM) as did urinary calcium (5.23 ± 0.37 *vs.* 3.57 ± 0.35 mmol/d, $P < 0.0001$, mean ± SEM). The high-protein diet caused a significant reduction in the fraction of urinary calcium of bone origin and a nonsignificant trend toward a reduction in the rate of bone turnover. There were no protein-induced effects on net bone balance. These data directly demonstrate that, at least in the short term, high-protein diets are not detrimental to bone. (*J Clin Endocrinol Metab* 90: 26–31, 2005)

MORE THAN 80 yr ago, Sherman (1) documented that humans fed a high-protein diet developed hypercalciuria. A recent meta-analysis of 26 controlled human intervention studies shows that for every 25-g increase in dietary protein, there is a 0.8 mmol rise in urinary calcium (2). The source of the additional urinary calcium excreted during a high-protein diet is unknown.

A popular hypothesis is that the additional calcium excreted in the urine results, in part, from changes in bone homeostasis. This hypothesis is supported by some but not all studies. It is well established that dietary protein increases endogenous acid production (3). In response to the acid load, bone may be called upon as a reservoir of alkali, and, as a consequence, bone calcium is mobilized. If true, then the long-term consequence of buffering by bone would be increased bone resorption and a decline in bone mineral density (BMD). Consistent with this hypothesis, osteoclast-mediated bone resorption is increased in an acid environment when compared with studies done at neutral or alkaline pH (4–7). Most balance studies (2) have reported that intestinal calcium absorption is unaffected by dietary protein, suggesting that the source of the extra urinary calcium resulting from a high-protein diet was not of intestinal origin. At odds with the bone hypothesis are a large number of epidemiological studies showing that a high-protein diet is associated with a high, not a low BMD, as might be predicted (8–17).

Because osteoporosis is a major public health problem, clarifying the impact of dietary protein on the skeleton is important. Therefore, we employed dual stable calcium isotopes to determine the relative contributions of the skeleton and the intestine to the rise in urinary calcium during the first week of a high-protein diet.

Subjects and Methods

Study design

The protocol consisted of two cycles involving 2 wk of an adjustment diet, followed by 10 d of an experimental diet. For the adjustment period, subjects modified their diets to contain moderate amounts of protein, sodium, calcium, and caffeine. During the 10-d experimental period, subjects received all food from the Yale General Clinical Research Center (GCRC) metabolic kitchen. These diets contained tightly controlled levels of calcium, sodium, phosphorus, and one of two levels of dietary protein: moderate (1.0 g/kg) or high (2.1 g/kg). The 2-wk adjustment period and 10-d experimental cycle were repeated again until all subjects received both levels of protein in random order. Fasting blood and urine samples were collected on the mornings of d 0 and 4 of each experimental period. During the morning of d 4, subjects received an iv dose of ^{42}Ca. Three oral doses of ^{44}Ca were administered with each meal during that day. For the next 6 d, blood and urine samples were collected for measurements of ^{42}Ca and ^{44}Ca.

Subjects

Ten healthy young women between 20–40 yr of age (29 ± 6 yr, mean ± SD) and three healthy postmenopausal women between 55–70 yr (61 ± 7 yr, mean ± SD) participated in the study. Body weight averaged 65 ± 2 and 65 ± 6 kg and body mass index averaged 23 ± 1 and 25 ± 2 kg/m^2 in the young and older women, respectively. Exclusion criteria included medications known to affect calcium metabolism, amenorrhea, pregnancy, smoking, eating disorders, diabetes, renal or gastrointestinal disease, bone disease, nephrolithiasis, or intensive daily

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Abbreviations: BMD, Bone mineral density; GCRC, General Clinical Research Center; RNAE, renal net acid excretion; TRCa, tubular reabsorption for calcium; Vbu, urinary calcium derived from bone; Vo+, bone calcium deposition; Vo−, bone calcium resorption; Vou, urinary calcium derived from the recent diet.

physical exercise. Subjects suspended all vitamin and mineral supplements during the entire study.

The racial background of the subjects was either Caucasian or Asian because these are the groups of women at highest risk for osteoporosis. Subjects continued their usual activities at home, school, and work during the study. The study was approved by Investigational Review Boards at Yale University (New Haven, CT), the University of Connecticut (Storrs, CT), and Johns Hopkins University (Baltimore, MD). Informed consent was obtained from each participant.

Diets

The experimental and adjustment diets are similar to those described in previous reports (18, 19). During the first week of the adjustment period, subjects were instructed to select diets containing approximately 1.2 g protein/kg, 20 mmol calcium, and 100 mmol sodium. This diet was provided to them by the GCRC's metabolic kitchen during the second week of the adjustment period to completely control nutrient intake. Subjects consumed adequate energy for weight maintenance. Caffeine-containing beverages were limited to one a day, and alcohol was not permitted.

During the 10-d experimental period, subjects reported daily to the GCRC to receive their meals and record their body weight. Energy intake ranged from 0.13 to 0.15 MJ/kg (30–35 kcal/kg) and was adjusted with simple sugars and fats to maintain body weight. We asked that the subjects consume all the food and beverage (and nothing other than the food and beverage) that was provided to them. Distilled water was provided ad lib.

All experimental diets were individually calculated to contain one of two levels of protein, whereas the levels of other nutrients were fixed (20 mmol calcium, 100 mmol sodium, and 32–39 mmol phosphorus). The macronutrient and mineral composition of the experimental diets was calculated from the U.S. Department of Agriculture Handbook No. 8 and manufacturer's information. The primary sources of calcium in the experimental diets were dairy foods and a chewable calcium carbonate (Tums, GlaxoSmithKline, Pittsburgh, PA). The increase in protein from the control to the high-protein diet was accomplished by adding both animal and vegetable sources of protein. Renal net acid excretion (RNAE) was estimated by the method of Frassetto *et al*. (20). RNAE (mEq/d) = 62.1 × (protein in g/potassium in mEq) −17.9. Using their equation, the estimated RNAE resulting from our medium-protein diet was 40.5 ± 2.6 and high-protein diet was 109.4 ± 6.5 ($P < 0.0001$).

Nonisotopic sample collection and analyses

Fasting blood collections were obtained at d 0 and 4 of each 10-d experimental period for measures of PTH, total and ionized calcium, phosphorus, and creatinine. A timed 24-h urine was collected on d −1 and d 3 for measurement of calcium, phosphorus, sodium, and creatinine. The tubular reabsorption for calcium (TRCa) was calculated from a fasting 2-h morning urine collection (0700–0900 h) and a midpoint blood sample (0800 h) using serum creatinine and ionized calcium and urine calcium and creatinine. The mean d 4 TRCa on the medium diet was 0.978 ± 0.006 and on the high diet 0.976 ± 0.003 (not significant). All nonisotopic assays were performed as reported (18).

Isotope administration and sample analysis

On d 4 of the experimental diet, oral ^{44}Ca was administered in three divided doses, delivered with each meal in proportion to the calcium content of the meal. Dividing the oral dose corrects for potential differences in calcium bioavailability between meals. Each oral calcium isotope was equilibrated in milk for 8–24 h. Immediately after breakfast, the subject received an iv infusion of ^{42}Ca, administered over a 5-min interval. The IV line was then flushed with saline. Immediately after the IV infusion, eight blood samples were collected at 5, 10, 20, 40, 60, 120, 240, and 480 min. All urine was then collected in acid-washed containers for the next 34 h in pools of 8, 12, and 14 h. Three spot urine samples were collected daily by the subject beginning the evening of d 5 and continuing until the end of d 9.

Calcium isotope ratios were measured using either a quadrupole (THQ, Finnigan, Bremen, Germany) or magnetic sector (Triton TI, Finnigan, Bremen, Germany) thermal ionization mass spectrometer. A ratio was made between each administered calcium tracer (^{42}Ca and ^{44}Ca) and another naturally occurring calcium isotope (^{48}Ca or ^{43}Ca). All isotopes were corrected for isotopic fractionation by normalizing the data to the $^{43/48}$Ca or $^{48/43}$Ca. Fractional calcium absorption was determined as the ratio of the cumulative oral tracer recovery to the cumulative IV tracer recovery in the 34-h urine collection obtained postdosing. True calcium absorption was calculated as the product of fractional calcium absorption and the calculated calcium intake.

Calcium kinetic measurements were determined using a multicompartmental model of calcium kinetics (21–23). After the first 24 h, the clearance of the oral and IV calcium tracer parallel one another and urine samples can be used to monitor the disappearance of the IV tracer. Rates of bone calcium deposition (Vo+) and resorption (Vo−) were then determined after accounting for urinary and endogenous fecal calcium losses. Net bone balance was determined as the difference between Vo+ and Vo−. The relative contribution of the recent diet and bone to urinary calcium excretion was calculated by the method of Welch *et al.* (24). Urinary calcium derived from the recent diet (Vou) is the product of dietary intake and the percentage of oral isotopic calcium recovered in the urine. Urinary calcium derived from bone (Vbu) is the difference between urinary calcium and Vou.

Statistical analyses

All values are presented as mean ± SEM (unless otherwise specified). A Student's *t* test was initially used to compare the younger with the older women, and because there were no important differences in calcium homeostasis, the two age groups were pooled for all subsequent analyses. A paired Student's *t* test was then used to evaluate differences in all biochemical outcomes between the two levels of protein at baseline and at d 4. The mean dietary intakes were evaluated between the two levels of protein using a paired Student's *t* test. A probability level of $P < 0.05$ was statistically significant.

Results

Subjects remained healthy throughout the experiment. The average nutrient composition of the experimental diets is presented in Table 1. As shown in Table 2, the average urinary sodium on d 4 of the moderate- and high-protein diets was not different and indicative of good compliance.

Baseline measures (d 0) of serum and urine metabolites did not differ between the two diets (Table 2). Aside from urinary calcium, the diets induced no changes in calcium indices that we measured at d 4 (Table 2). By d 4, urinary calcium in every subject increased during the high-protein diet (Fig. 1), rising from 3.57 ± 0.35 during the moderate to 5.23 ± 0.37 mmol/d during the high-protein diet ($P < 0.0001$).

TABLE 1. Calculated mean daily nutrient composition of experimental diets[a]

	Moderate protein (1.0 g/kg)	High protein (2.1 g/kg)
Protein (g)	66.5 ± 2.3	136.4 ± 4.9
Energy (MJ)	8.56 ± 0.28	8.91 ± 0.23
Carbohydrate (g)	304 ± 12	250 ± 10[b]
Fat (g)	63 ± 3	66 ± 4
Calcium (mmol)	20.0 ± 0.0	20.0 ± 0.2
Phosphorus (mmol)	34.7 ± 0.7	37.9 ± 0.3[c]
Magnesium (mmol)	11.3 ± 0.4	11.3 ± 0.3
Sodium (mmol)	100.0 ± 0.1	99.8 ± 0.2
Potassium (mmol)	71.5 ± 2.5	67.5 ± 2.3
Fiber (g)	20.5 ± 0.6	16.0 ± 0.9[b]

[a] Mean ± SEM on d 4 (n = 13). Statistical differences between protein intakes are not reported because these were the sorting variables.

[b] Significantly different from the moderate protein level, $P < 0.001$.

[c] Significantly different from the moderate protein level, $P < 0.01$.

TABLE 2. Effect of dietary protein on calcium-related metabolites

Day	Moderate protein (1.0 g/kg)		High protein (2.1 g/kg)	
	0	4	0	4
Serum metabolites				
Total Ca (mmol/liter)	2.41 ± 0.02	2.30 ± 0.03	2.39 ± 0.03	2.30 ± 0.03
Ionized Ca (mmol/liter)	1.22 ± 0.01	1.21 ± 0.01	1.21 ± 0.01	1.20 ± 0.01
Phosphorus (mmol/liter)	1.19 ± 0.04	1.19 ± 0.04	1.20 ± 0.03	1.14 ± 0.04
PTH (nmol/liter)	14.4 ± 0.4	15.4 ± 0.5	14.6 ± 0.4	15.0 ± 1.8
24-h urine				
Sodium (mmol)	108 ± 9	106 ± 8	99 ± 6	119 ± 7
Calcium (mmol)	3.97 ± 0.32	3.57 ± 0.35	4.04 ± 0.40	5.23 ± 0.37[a]
Phosphorus (mmol)	21.9 ± 1.7	21.1 ± 1.2	20.3 ± 1.4	22.8 ± 1.6
GFR (ml/min)	110 ± 6	113 ± 6	113 ± 6	118 ± 7

Data are presented as mean ± SEM on d 0 and 4 (n = 13). GFR, Glomerular filtration rate.
[a] Significantly different from the moderate protein level within the same day, $P < 0.0001$.

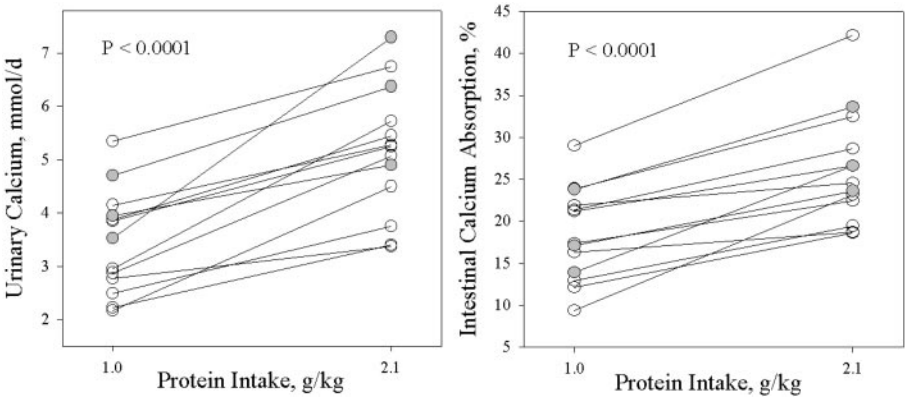

FIG. 1. Urinary calcium (*left panel*) and intestinal calcium absorption (*right panel*) at d 4 on the moderate (1.0 g/kg) and high-protein (2.1 g/kg) diets. Each *line* represents an individual subject. The *gray circles* represent the three postmenopausal women, whereas the 10 *open circles* represent the young women. The *P* value identifies the statistical difference between levels of protein.

There were no statistically significant differences in kinetic measures of bone formation, resorption, or balance between the diets (Table 3). However, there was a nonsignificant trend toward lower bone turnover (both formation and resorption) during the high-protein diet in comparison with the moderate control protein. There was a significant increase during the high-protein diet in the relative fraction of urinary calcium of dietary origin and a significant decrease in the relative fraction of urinary calcium from bone origin compared with the moderate protein diet (Table 3). All subjects were in negative calcium balance during all interventions.

Percent intestinal calcium absorption increased during the high-protein diet (26.2 ± 1.9%) in comparison with the moderate (18.5 ± 1.6%, $P < 0.0001$), representing a mean increase of 7.7% (raw increase) or 42% (relative increase; Fig. 1). Because dietary calcium was fixed at 20 mmol/d, the intestinal absorption on the high-protein diet was 1.54 ± 0.20 mmol/d higher than on the moderate diet. The mean increase in urinary calcium between the two diets was 1.66 ± 0.25 mmol/d.

Discussion

We used dual stable calcium isotopes to quantify the contributions of bone and intestine to the increment in urinary calcium observed during a high-protein diet. High-protein diets (in comparison with the moderate, control protein diet) resulted in a significant increase in intestinal calcium absorption that paralleled the increase in urinary calcium excretion. These effects were evident in all subjects and were not influenced by age across the age range studied. The increase in true calcium absorption during the high-protein diet was accompanied by a significant decrease in the fraction of calcium from nondietary (bone) origin. Therefore, at least acutely, the increase in urinary calcium from a high-protein diet is explained by increases in intestinal calcium absorption with no contribution from bone resorption. Finally, no matter what level of protein was ingested, all women were in negative calcium balance while consuming the 20 mmol calcium, suggesting that 20 mmol calcium is not adequate to maintain bone balance.

A regression equation based on data from 26 published human studies (that included a wide variety of protein sources, subjects, and study design) predicts that a 58-g in-

TABLE 3. Effect of dietary protein on d 4 kinetic measures of calcium absorption and bone turnover

	Moderate protein (1 g/kg)	High protein (2.1 g/kg)
Intestinal Ca absorption (%)	18.5 ± 1.6	26.2 ± 1.9[a]
Vo+ bone formation (mmol Ca/d)	15.1 ± 1.4	12.3 ± 1.4
Vo−, bone resorption (mmol Ca/d)	17.9 ± 1.4	14.8 ± 1.3
Bone balance (mmol Ca/d)	−2.8 ± 0.4	−2.5 ± 0.4
Vou (% of urine Ca from diet)	4.0 ± 0.4	5.6 ± 0.5[a]
Vbu (% of urine Ca from bone)	96.0 ± 0.4	94.4 ± 0.5[a]

Data are presented as mean ± SEM, n = 13.
[a] Significantly different from the moderate protein level, $P < 0.0001$.

crease in dietary protein would result in a 1.86 mmol rise in urinary calcium (2). Consistent with this prediction, the addition of 58 g of protein to the diets of our subjects increased urinary calcium by 1.66 mmol.

The dual stable calcium isotope method we used measures true intestinal calcium absorption. Although we did not measure fecal calcium and cannot directly quantify endogenous excretion of calcium, we can estimate endogenous calcium excretion based on published equations (25). Intestinal calcium secretion can itself be influenced by calcium absorption and dietary phosphorus. During the high-protein diet, when absorption efficiency is increased, one would expect that both dietary calcium and endogenously secreted calcium would be absorbed more efficiently, so there would be less calcium in the feces. We have calculated, on the basis of published equations (25), that the 7.7% raw increase in intestinal calcium absorption would result in a decrease of 0.2 mmol/d in endogenous fecal calcium excreted during the high-protein diet. However, the high-protein diet contained 3.2 mmol more phosphorus than the medium-protein diet. Again, on the basis of the work of Heaney and Recker (25), this difference in dietary phosphorus would lead to an additional 6 mg (0.2 mmol) calcium in the intestinal tract during the high-protein diet. Thus, these two countervailing effects negate each other because the increased absorption of secreted calcium is off set by the dietary phosphorus-induced increase in endogenous fecal calcium. Furthermore, it should be kept in mind that these calculated changes are relatively small in relation to the large change in urinary calcium between the diets of 1.66 mmol.

The 42% relative increase (or 7.7% raw increase) in intestinal calcium absorption (18.5 *vs.* 26.2%) in the current study accounts for 1.54 of the 1.66 mmol rise in urinary calcium or approximately 93%. The difference in intestinal calcium absorption between the control and the high-protein diet in our study is large and probably explains why human intervention studies generally report an increase in urinary calcium in response to a high-protein diet despite variability in experimental design, subjects, types of diets, and nutrient composition (2).

It is often suggested that a decrease in renal tubular calcium reabsorption explains, in part, the hypercalciuria observed on a high-protein diet. This was not the case in our experiment because TRCa was unchanged on the two diets (medium diet, 0.978 ± 0.006; high diet, 0.976 ± 0.003; P = not significant).

The majority of the balance studies in humans have not reported a change in calcium absorption in response to dietary protein (2). However, there are at least three balance studies that showed an increase in calcium absorption as dietary protein is increased (26–28). In general, balance studies should be interpreted with caution because the method is highly dependent on quantifying fecal calcium loss, which is difficult to measure accurately.

Two isotopic studies found no association between dietary protein intake and calcium absorption (29, 30). Heaney (29) reported that, in a longitudinal study of a large group of Roman Catholic nuns (mean age 48.7 ± 7.0 yr), there was no relationship between intestinal calcium absorption and usual intake of dietary protein. The mean protein intake in his study was 62 g (or approximately 1 g/kg), and their calcium intake averaged 17.6 mmol/d. Dawson-Hughes found similar results in adults over the age of 65 yr (30). Although these studies employed isotopic measures of calcium absorption, a method generally considered more accurate than a balance approach, dietary protein was not experimentally manipulated. It is possible that the large interindividual variability in intestinal calcium absorption and the multitude of other dietary factors that can also affect calcium absorption may explain why the relationship between protein intake and intestinal calcium absorption was not observed in these two studies.

In general, the evidence that dietary protein influences intestinal calcium transport in animals is strong. In rodents, several studies have shown that increasing dietary protein increases intestinal calcium absorption and shifts the route of calcium excretion from the intestinal tract to the urine (31–33).

The mechanism that underlies the rise in intestinal calcium absorption in response to dietary protein is unknown. Dietary protein and amino acids are gastric acid and gastrin secretagogues (34, 35). Gastric acid helps to solubilize dietary calcium and could potentially improve its bioavailability, although the literature on this topic is contradictory (36–38). Alternatively, the peptide fragments (*e.g.* casein phosphopeptides) that are released during dairy protein digestion may aid in solubilizing calcium, making it more available for absorption (39–42).

Although not statistically significant, there was a trend toward decreased bone turnover (Table 2) during the high-protein diet. The high-protein diet was associated with a significant decrease in the fraction of urinary calcium that was of bone origin. We calculated RNAE by the method of Frassetto *et al.* (20) to estimate the potential renal acid load generated by the two experimental diets. The two are highly correlated (20). By these calculations, the high-protein diet generated 2.7 times more fixed acid than did the control diet (RNAE in mEq/d was 109.4 ± 6.5 high protein *vs.* 40.5 ± 2.6 medium protein; $P < 0.0001$). Despite this, there was no effect on bone resorption.

Our experimental intervention of 1 wk was perhaps too short to detect significant changes in bone homeostasis despite the large change in intestinal calcium absorption. Moreover, because of the variability in rates of bone turnover, larger sample sizes may be needed to detect significant changes in this parameter even with a paired study design. However, the acute increase in intestinal calcium absorption and trend toward decreased bone turnover observed in our study may help explain the epidemiological studies that show higher BMD associated with high protein intakes (8–17). Clearly, longer term intervention studies are needed to answer the question.

In a recent isotopic intervention study of 8 wk in length, Roughead *et al.* (43) evaluated calcium retention using sensitive radiotracer and whole-body scintillation counting in 15 postmenopausal women as they consumed moderate and high-protein diets. Calcium retention did not differ between the two levels of protein, although there was a nonsignificant trend toward better retention during the high-protein diet. Taken together, the two isotopic

intervention studies [ours and that of Roughead *et al*. (43)] support the conclusion that high-protein diets, at least in the short and intermediate term, do not result in increased bone loss. If anything, both studies showed nonsignificant trends toward lower bone turnover and better calcium retention during high-protein diets.

Our finding that the average bone balance in our subjects was negative by 2.5 mmol/d (no matter what level of dietary protein was consumed) was unanticipated. No subject was in positive bone balance on any intervention, even when intestinal calcium absorption was highest during the high-protein diet. Clearly, 20 mmol dietary calcium is not enough to maintain calcium balance in our healthy female adults, both young and old. We selected 20 mmol calcium because it was the recommended daily allowance at the time the study was designed, and it approximates the calcium intake of many adult women. In the most recent version of the Dietary Reference Intakes, recommendations are higher, 25–30 mmol, depending on age (44). It would be important to determine whether the current higher recommended intakes for calcium would restore bone balance.

In summary, we used dual stable calcium isotopes to evaluate the acute impact of dietary protein on bone kinetics in a diet-controlled, cross-over study. The majority of the rise in urinary calcium in response to an increase in dietary protein was due to an increase in intestinal calcium absorption. Our short-term study needs to be extended to longer term intervention trials to fully evaluate the impact of dietary protein on bone turnover. Our study does not support the hypothesis that increased dietary protein is acutely harmful to bone.

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