A threshold for low-protein-diet–induced elevations in parathyroid hormone[1–3]

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ABSTRACT

Background: We reported previously that lowering dietary protein intake in young healthy women to 0.7 g/kg depressed intestinal calcium absorption and was accompanied by elevations in parathyroid hormone (PTH). Moderate amounts of dietary protein (1.0 g/kg) did not appear to perturb calcium homeostasis.

Objective: The purpose of this study was to evaluate the effect of graded intakes of dietary protein (0.7, 0.8, 0.9, and 1.0 g/kg) on calcium homeostasis.

Design: The experiment consisted of 2 wk of a well-balanced diet containing moderate amounts of calcium, sodium, and protein followed by 4 d of an experimental diet containing 1 of 4 amounts of protein. Eight young healthy women received the 4 amounts of protein in random order. The average age of the subjects was 23.1 ± 2.3 y, their weight was 64 ± 3 kg, and their body mass index (in kg/m^2) was 24.3 ± 0.9.

Results: Elevations in PTH developed by day 4 of the diets containing 0.7 and 0.8 g protein/kg but not during the diets containing 0.9 or 1.0 g protein/kg. By day 4 of the 0.7- and 0.8-g/kg diets, midmolecule PTH, calcitriol, and nephrogenous cyclic adenosine monophosphate were 1.5–3.5-fold higher than on day 0. Calcitropic hormones on day 4 of the diets containing 0.8 and 0.9 g protein/kg were within the normal range and 23–57% lower than values observed with the 0.7- and 0.8-g/kg diets ($P < 0.005$). Mean 24-h urinary calcium was 3.29 ± 0.35 mmol with the diet containing 0.7 g protein/kg and 3.54 ± 0.46 mmol with the diet containing 1.0 g protein/kg.

Conclusions: Our data suggest that in young healthy women consuming a well-balanced diet, the current recommended dietary allowance for protein (0.8 g/kg) results in short-term perturbations in calcium homeostasis. *Am J Clin Nutr* 2000;72:168–73.

KEY WORDS Dietary protein, calcium metabolism, calcitropic hormones, vitamin D, parathyroid hormone, young healthy women, recommended dietary allowance

INTRODUCTION

The effect of dietary protein on calcium metabolism in humans was first documented nearly 80 y ago (1). There has been considerable investigative interest in the potential negative effects of a high-protein diet on mineral and skeletal homeostasis. For example, increasing dietary protein induces urinary calcium loss, negatively affects calcium balance (2), increases bone turnover (3), and may be associated with an increased risk of fracture (4). However, the effect of low-protein diets on calcium and bone homeostasis has received considerably less attention.

We reported that in 16 young healthy women, 4 d of a low-protein diet decreased urinary calcium excretion and was accompanied by elevations in calcitropic hormones; 1.5–3-fold increases were observed in concentrations of serum parathyroid hormone (PTH), 1,25(OH)$_2$vitamin D (calcitriol), and urinary nephrogenous cyclic adenosine monophosphate (NcAMP; a bioindex of PTH action). The low-protein diet contained an average of 45 g protein (0.7 g/kg), including both animal and vegetable sources of protein; 20 mmol calcium; and 100 mmol sodium and was otherwise well balanced (5). The rise in PTH appeared to be due, in part, to a significant reduction in intestinal calcium absorption as measured by dual stable calcium isotopes (6). Intestinal calcium absorption averaged 26 ± 3% during a high-protein intake (2.1 g/kg) and decreased to 19 ± 3% when protein was restricted to 0.7 g/kg (6). Consistent with other reports (7), we detected no alterations in mineral homeostasis at a protein intake of 1.0 g/kg (5).

The current recommended dietary allowance (RDA) for dietary protein in young women is 0.8 g/kg (8), an amount that lies between 0.7 g/kg (which reduces intestinal calcium absorption and stimulates the PTH–1-α-hydroxylase axis) and 1.0 g/kg (at which calcium homeostasis appears to be normal). The purpose of this study was to evaluate the effect of graded intakes of dietary protein (0.7, 0.8, 0.9, and 1.0 g/kg) on calcium metabolism to determine whether there was a threshold or a linear

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relation between moderately low dietary protein and parathyroid function. Our long-term goal is to determine the minimal optimal amount of dietary protein required for normal calcium metabolism in young healthy women.

SUBJECTS AND METHODS

Study design

The study design was similar to that described previously (5). The protocol consisted of 4 cycles involving 2 wk of an adjustment diet, followed by 4 d of an experimental diet, and 3 d of an ad libitum diet. For the adjustment period, the subjects were instructed to modify their usual dietary intakes to contain moderate amounts of protein (≈1 g/kg), sodium (≈100 mmol), calcium (≈20 mmol), and caffeine (1 caffeine-containing beverage/d). During the 4-d experimental periods, the subjects received all food from the Yale General Research Center's metabolic kitchen. The diets contained tightly controlled amounts of calcium, sodium, and phosphorus and 1 of 4 graded amounts of dietary protein: 0.7, 0.8, 0.9, or 1.0 g/kg. Fasting blood and urine samples were collected on the mornings of days 0 and 4 of each experimental period. The 2-wk adjustment period and 4-d experimental cycle were repeated 3 more times until all the subjects received all amounts of protein in random order.

Subjects

Eight healthy women aged 20–40 y were recruited to participate in the study. The average age of the study subjects was 23.1 ± 2.3 y, their weight averaged 64 ± 3 kg, and the average body mass index (in kg/m^2) was 24.3 ± 0.9. Potential subjects were initially interviewed by telephone to obtain basic health information. Exclusion criteria included use of medications known to affect calcium metabolism (eg, glucocorticoids, nonsteroidal antiinflammatory medications, vitamin D, and birth control pills); amenorrhea; pregnancy; and a history of smoking, eating disorders, diabetes, renal or gastrointestinal disease, bone disease, nephrolithiasis, and intensive daily physical exercise. The subjects were asked to suspend any vitamin or mineral supplementation during the entire study. The races of the subjects were white and Asian. The subjects were free-living and continued their usual activities at home, school, and work during the study. Informed consent was obtained from each participant. The study was approved by Human Investigation Committees at both Yale University and the University of Connecticut.

Diets

The experimental and adjustment diets were similar to those described previously (5). The subjects were instructed by the research dietitian to self-select their adjustment diets to contain ≈1 g protein/kg, 20 mmol Ca, and 100 mmol Na. The subjects consumed energy for weight maintenance. Caffeine-containing beverages were limited to one per day and alcohol was not permitted.

During the 4-d experimental period, the subjects reported daily to the General Research Center to receive their meals and record their body weights. 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. Average body weight fluctuated by <1% during the 4-d experimental periods.

All experimental diets were individually calculated to contain 1 of 4 amounts of protein: 0.7, 0.8, 0.9, and 1.0 g/kg, whereas other nutrients remained controlled (19.8–20.3 mmol Ca, 99–101 mmol Na, and 26–36 mmol P). The subjects used low-sodium herbs, spices, condiments, seltzer water, and distilled drinking water ad libitum. The macronutrient and mineral compositions of the experimental diets was calculated by using the US Department of Agriculture *Handbook no. 8* (9) and manufacturers' information.

Protein quality was held relatively constant among the 4 diets by routinely including meat or dairy products in all experimental menus. The increment in protein between the experimental diets was made by adding equal amounts of animal and vegetable protein. For example, of the total 6-g difference between adjacent amounts of dietary protein, ≈3 g was from animal and ≈3 g from vegetable sources. The amino acid content of each experimental diet was calculated by using FOOD PROCESSOR PLUS (version 6.01; ESHA Research, Salem, OR). The primary sources of calcium in the experimental diets were dairy foods and a commercially available, chewable form of calcium carbonate (Tums; SmithKline Beecham, Pittsburgh).

Sample collection and analyses

Blood and urine samples were collected at the beginning and end of each 4-d experimental period. A timed, 24-h urine sample was collected by the subjects on days −1 and 3 for measurement of calcium, phosphorus, sodium, and creatinine. Twenty-four–hour urinary nitrogen excretion was measured at the end of each experimental period. Fasting 2-h urine samples were obtained on days 0 and 4 to measure NcAMP and creatinine excretion. Blood was drawn at the midpoint of the 2-h period for measurement of midmolecule PTH, calcitriol, total and ionized calcium, phosphorus, and creatinine.

Assays

All assays were performed as previously reported (5). Briefly, serum and urinary creatinine, urinary sodium, and urinary total nitrogen were measured in the Clinical Chemistry Laboratories of the Yale New Haven Hospital. Serum total and urinary calcium were measured by using flame-atomic absorptiometer (model 2380; Perkin Elmer, Norwalk, CT). Blood ionized calcium was measured in a nondiluted sample by using a Beckman Lablyte 820, an ion-selective electrode (Beckman, Brea, CA).

Intact PTH (1–84) was measured with use of a 2-site immunoradiometric assay (Allegro Intact PTH; Nichols Institute, San Juan Capistrano, CA) and midmolecule PTH was measured with antiserum to the midregion of human PTH, ^{125}I-labeled bovine PTH (37–84) as radioactive trace, and standards from a human PTH adenoma extract. The method of Reinhardt et al (10) was used to measure serum calcitriol. Plasma and urinary cyclic AMP were measured as previously reported (11). NcAMP was calculated from plasma and urinary cyclic AMP measurements (11, 12).

Statistical analysis

All values are presented as means ± SEMs. Repeated-measures analysis of variance was used to evaluate differences between the 4 amounts of protein at baseline (day 0) and at day 4. A probability level of $P < 0.05$ was considered statistically significant. Comparisons were made between adjacent amounts of protein (0.7 compared with 0.8, 0.8 compared with 0.9, and 0.9 compared with 1.0) by using post hoc orthogonal contrasts and a Bonferroni correction for multiple tests (13).

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

	Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)			
	0.7	0.8	0.9	1.0
Protein				
(g)	44.3 ± 1.9	50.2 ± 2.4	56.7 ± 2.6	62.7 ± 2.8
(g/kg)	0.7 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
Energy (MJ)	9.22 ± 0.37	8.94 ± 0.41	8.55 ± 0.35	8.46 ± 0.34
Carbohydrate (g)	372 ± 16	358 ± 14	331 ± 16[2]	323 ± 17
Fat (g)	60 ± 4	56 ± 5	55 ± 3	53 ± 2
Calcium (mmol)	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0
Phosphorus (mmol)	29.3 ± 0.8	30.8 ± 1.1	31.6 ± 0.9	33.5 ± 0.9[2]
Magnesium (mmol)	10.5 ± 0.3	10.5 ± 0.5	10.6 ± 0.4	10.8 ± 0.7
Sodium (mmol)	100.0 ± 0.2	100.1 ± 0.1	100.0 ± 0.2	99.8 ± 0.1
Potassium (mmol)	64.5 ± 3.4	63.4 ± 3.4	66.5 ± 4.3	64.9 ± 5.5
Fiber (g)	17.0 ± 1.2	17.1 ± 1.3	16.7 ± 1.5	17.4 ± 1.3
Amino acid (g)				
Histidine	1.09 ± 0.05	1.29 ± 0.06[3]	1.43 ± 0.06[3]	1.60 ± 0.07[3]
Isoleucine	1.93 ± 0.09	2.26 ± 0.10[3]	2.54 ± 0.11[3]	2.84 ± 0.13[3]
Leucine	3.27 ± 0.14	3.78 ± 0.15[3]	4.29 ± 0.17[3]	4.76 ± 0.21[3]
Lysine	2.43 ± 0.17	2.99 ± 0.16[3]	3.38 ± 0.18[2]	3.85 ± 0.20[3]
Methionine	0.91 ± 0.06	1.09 ± 0.06[3]	1.24 ± 0.06[2]	1.40 ± 0.07[3]
Phenylalanine	1.96 ± 0.07	2.28 ± 0.08[3]	2.53 ± 0.09[3]	2.81 ± 0.12[3]
Threonine	1.56 ± 0.09	1.83 ± 0.09[3]	2.07 ± 0.09[3]	2.32 ± 0.11[3]
Tryptophan	0.49 ± 0.02	0.58 ± 0.02[3]	0.64 ± 0.03[2]	0.71 ± 0.03[3]
Valine	2.30 ± 0.09	2.63 ± 0.10[3]	2.93 ± 0.11[3]	3.26 ± 0.13[3]

[1] $\bar{x}$ ± SEM on day 4; n = 8. Statistical differences between protein intakes are not reported because these were the sorting variables.
[2] Significantly different from the preceding lower protein intake, $P < 0.05$.
[3] Significantly different from the preceding lower protein intake, $P < 0.005$.

RESULTS

All the subjects remained healthy during the experiment. One subject failed to consume the 0.9 g/kg protein intake. The average nutrient and amino acid compositions of the experimental diets are presented in **Table 1**. As protein intake increased, carbohydrate and fat intakes gradually declined (although only carbohydrate intake differed significantly between the 0.8- and 0.9-g/kg intakes). Dietary phosphorus intake rose concurrent with dietary protein. The 10–15% rise in total dietary protein between the 4 intakes was mirrored by similar incremental increases in individual amino acid intakes.

Mean changes in mineral metabolism and calcitropic hormones are presented in **Table 2** and individual changes in calcitropic hormones are plotted in **Figure 1**. There were no significant differences between the 4 protein intakes in serum minerals or urine analytes measured on day 0 or day 4.

At the end of 4 d at the 2 lowest protein intakes, mean circulating concentrations of midmolecule PTH were 1.8–2.2-fold above the upper limit of normal. Furthermore, mean circulating concentrations of calcitriol were at the upper limit of normal at the 0.7-g/kg (145.7 ± 8.2 pmol/L) intake and high-to-normal at the 0.8-g/kg (128.7 ± 4.9 pmol/L) intake. In contrast, as is apparent from Table 2 and Figure 1, all measures of the PTH-1-α-hydroxylase axis remained well within the normal range on day 4 of the 0.9- and 1.0-g/kg intakes.

When mean concentrations of calcitropic hormones were compared between the 0.8- and 0.9-g/kg intakes of dietary protein, a dramatic decrease in circulating concentrations was apparent (Table 2 and Figure 1). Thus, for midmolecule PTH, mean concentrations were 57% higher at the 0.8-g/kg intake than at the 0.9-g/kg intake; similar large increments were noted in intact PTH (48%), calcitriol (25%), and NcAMP (23%).

DISCUSSION

This study showed that consumption of moderately low amounts of dietary protein (0.7 and 0.8 g/kg) stimulated the PTH-1-α-hydroxylase axis within 4 d in young healthy women consuming an otherwise well-balanced diet. By day 4 of the 0.7- and 0.8-g/kg intakes, midmolecule PTH, calcitriol, and NcAMP were 1.5–3.5-fold higher than at baseline. By contrast, midmolecule PTH values were within the normal range with the 0.9- and 1.0-g/kg intakes. Previous studies (3, 6) indicated that reduced intestinal calcium absorption and possibly a diminished rate of bone resorption underlie the abrupt changes in parathyroid function seen at a dietary protein intake of 0.7 g/kg. The results of the current study suggest (but do not prove) that similar changes may be occurring at a daily intake of 0.8 g/kg.

There were no significant differences in mean urinary calcium excretion over the relatively narrow range of dietary protein intakes studied, although the mean value with the 0.7-g/kg intake was lower than that with the 1.0-g/kg intake by 0.25 mmol (10 mg). The lack of change may be due to the small sample and the inherent variability in urinary calcium excretion. In a previous study involving 16 subjects, we found that the difference in 24-h urinary calcium excretion on day 4 between the 0.7- and 1.0-g/kg intakes was significant, albeit small [0.5 mmol (20 mg) Ca] (5).

The changes we observed in the calcitropic hormones in the current study paralleled those of our previous studies (5, 6).

TABLE 2
Effect of dietary protein on calcium-related metabolites[1]

	Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)							
	0.7		0.8		0.9		1.0	
	Day 0	Day 4	Day 0	Day 4	Day 0	Day 4	Day 0	Day 4
Serum minerals								
Total calcium (mmol/L)	2.38 ± 0.03	2.41 ± 0.04	2.42 ± 0.03	2.38 ± 0.04	2.40 ± 0.03	2.40 ± 0.02	2.34 ± 0.03	2.32 ± 0.03
Ionized calcium (mmol/L)	1.20 ± 0.01	1.22 ± 0.01	1.21 ± 0.01	1.21 ± 0.01	1.22 ± 0.02	1.23 ± 0.01	1.22 ± 0.01	1.22 ± 0.01
Phosphorus (mmol/L)	1.17 ± 0.05	1.21 ± 0.04	1.20 ± 0.05	1.18 ± 0.05	1.17 ± 0.02	1.20 ± 0.03	1.21 ± 0.02	1.20 ± 0.05
24-h urine								
Calcium (mmol)	4.29 ± 0.66	3.29 ± 0.35	4.55 ± 0.53	3.52 ± 0.44	4.48 ± 0.37	3.12 ± 0.33	3.79 ± 0.45	3.54 ± 0.46
Phosphorus (mmol)	22.9 ± 3.0	20.8 ± 2.0	26.5 ± 2.0	18.4 ± 0.8	26.1 ± 2.1	20.7 ± 2.0	26.1 ± 3.9	19.5 ± 1.6
Glomerular filtration rate (mL/min)	93 ± 12	99 ± 9	99 ± 9	93 ± 9	104 ± 10	95 ± 10	102 ± 10	94 ± 7
Sodium (mmol)	125 ± 13	102 ± 15	129 ± 18	89 ± 9	151 ± 14	94 ± 9	115 ± 9	103 ± 6
Nitrogen (mmol)		524 ± 52		541 ± 35		551 ± 55		581 ± 36
Calcitropic hormones								
Midmolecule parathyroid hormone (nmol/L)	12.2 ± 0.8	43.1 ± 2.6	12.3 ± 0.7	37.7 ± 1.4	12.1 ± 0.5	16.3 ± 0.1[4]	12.5 ± 0.5	15.0 ± 0.9
Intact parathyroid hormone (nmol/L)	2.0 ± 0.3	4.3 ± 0.4	1.8 ± 0.2	4.0 ± 0.4	1.7 ± 0.2	2.1 ± 0.2[3]	1.9 ± 0.2	2.2 ± 0.2
Calcitriol (pmol/L)	87.3 ± 4.3	145.7 ± 8.2	83.1 ± 3.9	128.7 ± 4.9	81.2 ± 2.6	95.9 ± 2.1[3]	85.1 ± 3.3	94.1 ± 3.7
NcAMP (nmol/L glomerular filtration)	14.1 ± 0.5	23.8 ± 1.2	13.9 ± 0.4	20.9 ± 0.9[2]	13.6 ± 0.3	16.1 ± 0.7[3]	14.2 ± 0.4	15.1 ± 0.4

[1] $\bar{x}$ ± SEM on days 0 and 4. NcAMP, nephrogenous cyclic adenosine monophosphate.
[2] Significantly different from day 4 of the 0.7-g protein intake, $P < 0.05$.
[3] Significantly different from day 4 of the 0.8-g protein intake, $P < 0.005$.
[4] Significantly different from day 4 of the 0.8-g protein intake, $P < 0.0001$.

Because the dietary protocol and diets in the current study were identical to those in the prior 2 studies, cross-study comparisons were possible. Thus, taking all 3 studies into account, the acute rise in PTH, NcAMP, and calcitriol (ranging from 25% to 250% between days 0 and 4) has now been observed in 31 young women studied while consuming a daily protein intake of 0.7 g/kg. The results of the current study add information regarding calcium metabolism at the 0.8- and 0.9-g/kg intakes.

The strikingly homogeneous individual responses in calcitropic hormones with the 4 diets are shown in Figure 1; the most consistent and largest decrement was between the 0.8- and 0.9-g/kg intakes. Giannini et al (14) reported a 22% increase in mean circulating concentrations of PTH in 18 middle-aged hypercalciuric men and women who had their dietary protein intakes lowered to 0.8 g/kg. Interestingly, serum PTH rose in that study despite a fairly high dietary calcium intake of 955 mg, an amount close to the current RDA (15). Both the Giannini et al study and ours raise the question of whether the current RDA for calcium (1000 mg) is adequate to overcome the rise in PTH observed when dietary protein is moderately restricted to 0.8 g/kg (the RDA for adults) (8).

The uniform decline in circulating concentrations of calcitropic hormones observed when the day 4 values at the 0.8-g/kg intake were compared with those with the 0.9-g/kg intake is intriguing. It suggests that, at least in this acute model, a dietary protein intake of 0.9 (and not 0.8) g/kg is the minimum required to ensure normal calcium homeostasis. Although we did not measure intestinal calcium absorption with the 0.8- and 0.9-g/kg intakes of protein, calcium absorption was low (19 ± 3%) (6) at the 0.7-g/kg intake and below the 25% generally expected in this population (16). Therefore, diminished intestinal calcium absorption may play a role in the observed changes in the PTH-1-α-hydroxylase axis. It would be of interest to determine directly whether intestinal calcium absorption was, in fact, significantly lower at the 0.8- than at the 0.9-g/kg intake.

Other than dietary protein, the minor differences in dietary macronutrients and minerals do not appear to explain the abrupt changes in the calcitropic hormones. The differences in dietary amino acid intakes (when expressed as gram intakes instead of amino acid ratios) (5), although small, may provide a potential explanation. For example, dietary lysine appears to increase intestinal calcium absorption in animals and humans; in rats, lysine enhances calcium deposition in the femur (17, 18). Mack et al (19) showed that lysine supplements in preadolescent children improved growth and bone density of the radius and os calcis relative to unsupplemented control subjects. However, the increment in lysine intake between the 0.8- and 0.9-g/kg intakes of protein (390 mg lysine) was approximately half the amount used by Civetelli et al (18) (800 mg) to induce changes in calcium absorption in postmenopausal women, so it is unlikely that lysine is the sole explanation for our findings. The precise mechanism by which low dietary protein impairs intestinal calcium absorption remains unknown.

The long-term consequences of restricted protein intake on calcium and bone metabolism are also unknown but could potentially be an important and unrecognized problem. Analysis of available data from the US Department of Agriculture indicated that 31% of women aged ≥20 y consume less protein than the 1989 RDA (20). Only half of these women (ie, 15% of women aged ≥20 y) considered their own diets to be too low in dietary protein (21). Preliminary reports from the Framingham Osteoporosis Study showed that low protein intakes are associated with low bone mineral density (22). A similar observation was made from the third National Health and Nutrition Examination Survey (NHANES III) database (23). Subclinical protein undernutrition may also induce bone loss after hip fracture. Bonjour et al (24) studied the effects of 6 mo of protein supplementation on bone loss in elderly subjects after hip fracture. The average dietary protein intake in that group was relatively low (≈40 g)

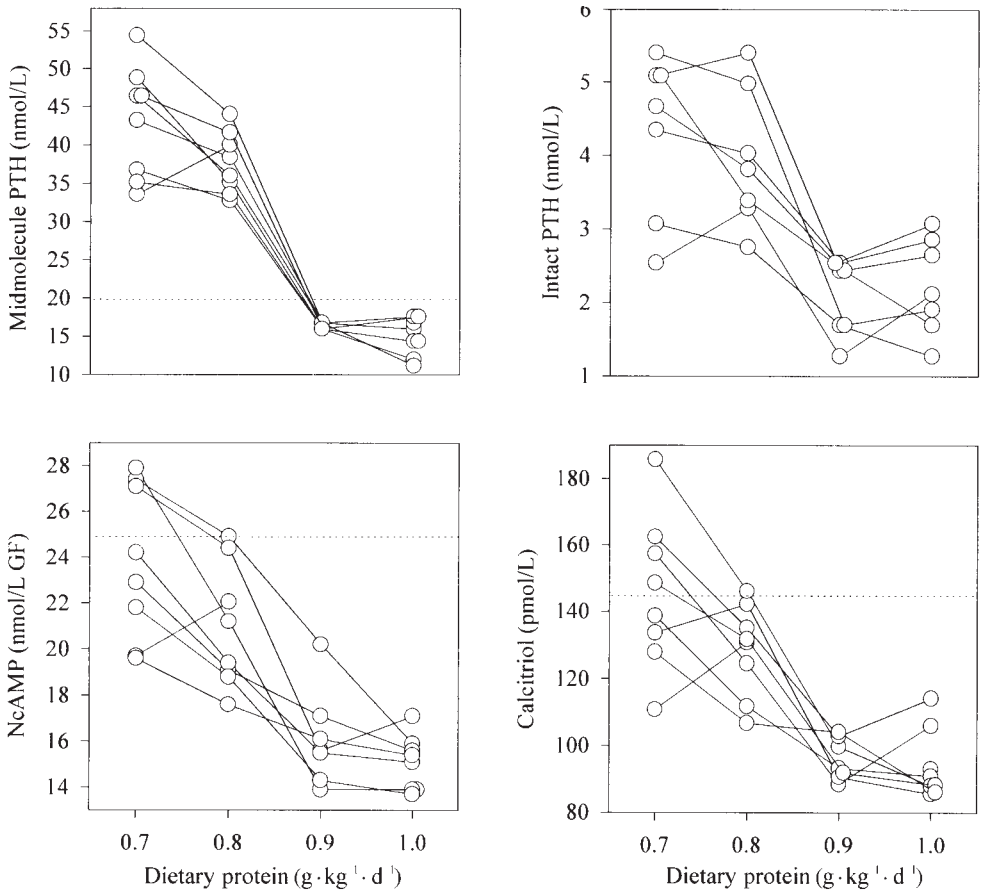

FIGURE 1. Individual responses in calcitropic hormones at day 4 in response to graded intakes of dietary protein (n = 8). The upper limits of normal are represented by horizontal dashed lines. For intact parathyroid hormone (PTH), n = 7. NcAMP, nephrogenous cyclic adenosine monophosphate; GF, glomerular filtrate

and supplementation with 20 g protein/d attenuated proximal femur bone loss by nearly 50% at 1 y.

In summary, we investigated the effect of graded intakes of dietary protein (0.7, 0.8, 0.9, and 1.0 g/kg) on calcium metabolism in young healthy women. Elevations in serum PTH developed within 4 d with the 2 lower intakes of protein, whereas calcitropic hormones remained within normal limits at the 0.9- and 1.0-g/kg intakes. These data suggest that in young healthy women consuming a well-balanced diet moderate in nutrients known to affect calcium metabolism, the RDA for protein (0.8 g/kg) does not support optimal calcium nutrition, at least acutely. Moderate protein intakes of ≥0.9 g/kg are needed to normalize calcium metabolism and prevent stimulation of the PTH–l-α-hydroxylase axis. More studies are needed to corroborate these potentially important findings.

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