The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

A Diet High in Meat Protein and Potential Renal Acid Load Increases Fractional Calcium Absorption and Urinary Calcium Excretion without Affecting Markers of Bone Resorption or Formation in Postmenopausal Women[1–4]

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Abstract

Our objective in this study was to determine the effects of a high-protein and high-potential renal acid load (PRAL) diet on calcium (Ca) absorption and retention and markers of bone metabolism. In a randomized crossover design, 16 postmenopausal women consumed 2 diets: 1 with low protein and low PRAL (LPLP; total protein: 61 g/d; PRAL: −48 mEq/d) and 1 with high protein and high PRAL (HPHP; total protein: 118 g/d; PRAL: 33 mEq/d) for 7 wk each separated by a 1-wk break. Ca absorption was measured by whole body scintillation counting of radio-labeled ^{47}Ca. Compared with the LPLP diet, the HPHP diet increased participants' serum IGF-I concentrations ($P < 0.0001$), decreased serum intact PTH concentrations ($P < 0.001$), and increased fractional ^{47}Ca absorption (mean ± pooled SD: 22.3 vs. 26.5 ± 5.4%; $P < 0.05$) and urinary Ca excretion (156 vs. 203 ± 63 mg/d; $P = 0.005$). The net difference between the amount of Ca absorbed and excreted in urine did not differ between 2 diet periods (55 vs. 28 ± 51 mg/d). The dietary treatments did not affect other markers of bone metabolism. In summary, a diet high in protein and PRAL increases the fractional absorption of dietary Ca, which partially compensates for increased urinary Ca, in postmenopausal women. The increased IGF-I and decreased PTH concentrations in serum, with no change in biomarkers of bone resorption or formation, indicate a high-protein diet has no adverse effects on bone health. J. Nutr. 141: 391–397, 2011.

Introduction

Although being essential to bone health, protein intake, especially from animal sources, in high amounts has also been considered a risk factor for osteoporosis or bone fractures (1–4) due to the increase in urinary calcium (Ca) excretion resulting from the metabolic acidity of protein metabolism (5–8). However, contrary to the assumption that high dietary protein impairs bone, many epidemiological observations link a high-protein intake with bone anabolism, including an association with increased bone mineral density or decreased fracture risk (9–14), with few reports showing negative associations (15,16).

The results of well-controlled human trials with Ca isotopes show that a high-protein intake increases intestinal Ca absorption (17–20). Whether such an increase in intestinal Ca absorption can offset hypercalciuria in a diet with a large difference in acid load remains unclear. Furthermore, the alleged detrimental effect of high-protein intake on bone may also be dependent upon other dietary factors, such as Ca intake (20).

Our previous finding that a diet with high compared with low meat protein did not adversely affect the retention of ^{47}Ca or induce calciurea (17) was questioned because of a relatively small difference (~32 mEq/d) in potential renal acid load (PRAL)[5] between the 2 diets (21). PRAL, as a measure of the

[1] Supported by the USDA Agricultural Research Service (ARS) program "Mineral Intakes for Optimal Bone Development and Health" Current Research Information System no. 5450-51000-039-00D as part of the authors' official duties. USDA, ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. This work was also partially funded by the Beef Check-off through the National Cattlemen's Beef Association (NCBA). The NCBA did not participant in the design, implementation, interpretation, or analysis of the research.
[2] Author disclosures: J. J. Cao, L. K. Johnson, and J. R. Hunt, no conflicts of interest.
[3] This trial was registered at clinicaltrials.gov as NCT00620763.
[4] Supplemental Figure 1 and Table 1 are available with the online posting of this paper at jn.nutrition.org
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[5] Abbreviations used: CTX, carboxyterminal cross-linking telopeptide; DPD, deoxypyridinoline; HPHP: high protein and high potential renal acid load; LPLP, low protein and low potential renal acid load; NTX, aminoterminal cross-linking telopeptide of bone collagen; OC, osteocalcin; OPG, osteoprotegerin; PRAL, potential renal acid load; PTH, parathyroid hormone; sRANKL, soluble receptor activator nuclear factor-κB ligand; TRAP, tartrate-resistant acid phosphatase.

Manuscript received July 17, 2010. Initial review completed August 23, 2010. Revision accepted November 30, 2010.
First published online January 19, 2010; doi:10.3945/jn.110.129361.

acid-base load of foods, can be used to estimate renal net acid excretion (22–24). Therefore, the objective of this controlled feeding study was to investigate how dietary Ca absorption and retention and urinary Ca excretion are influenced by high and low meat protein in convention diets with a substantial difference in PRAL, which spans the lowest and highest PRAL quintiles as estimated in a recent large cross-sectional study (25).

Participants and Methods

Study design. The study was reviewed and approved by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board as well as the USDA Radiological Safety Office. The study was explained verbally and in writing by the investigators and written informed consent was obtained from each participant.

Women consumed 2 experimental diets: low in protein and PRAL (LPLP) or high in protein and PRAL (HPHP). (The nomenclature "low protein" in the present context refers to differences between the 2 diets; the low-protein diet met nutritional recommendations for protein.) The diets were provided in random order for 7 wk each, with a 1-wk break between periods when diets were not controlled (15 wk total) (**Supplemental Fig. 1**). After 3 wk of dietary equilibration (e.g. at wk 4 and 11), an entire 2-d cycle menu of each diet was radio-labeled with ^{47}Ca. Ca absorption was measured by whole body scintillation counting for 4 wk after ingestion of the radio-labeled meals. Blood and urine samples were collected at several time points (Supplemental Fig. 1) to assess the effects of the diet on biomarkers of bone metabolism and renal adaptation to the acid load.

Participants. Healthy, nonsmoking postmenopausal women were recruited through public postcards and television, radio, print, and internet advertisements. The women were qualified to enter the study if they met the following selection criteria: age 40–75 y; at least 3 y since last menses; follicle stimulating hormone $>$ 40 IU/L; BMI within the 5th and 95th percentiles for their age; no apparent underlying disease as determined by a screening physical examination and blood tests (normal thyroid, liver, heart, and kidney functions); and no osteoporosis as indicated by bone mineral density T scores ≤ -2.5 for femoral neck region or for total spinal region (L1-L4) as determined by dual-energy X-ray absorptiometry (Hologic Delphi QDR); no regular use of medications; willing to discontinue any nutritional supplements and any Ca-containing medications (e.g. Ca-carbonate antacids) or any medications known to interfere with Ca and bone metabolism for the duration of the study; and an agreement not to use tanning booths. The participants were advised to keep normal leisure, household, and occupational activity.

Baseline characteristics of the study participants are shown in **Table 1**.

Diets. During the two 7-wk diet periods, all food and beverages were provided as weighed, controlled diets in a 2-d menu cycle (**Supplemental Table 1**). Diets were planned by registered dietitians based on USDA food composition data (26). Nutrient composition and the PRAL values for the diets are shown in **Table 2**. The PRAL values were calculated using the following formula: PRAL (mEq/d) = (mg P/d $\times$ 0.0366) + (g protein/d $\times$ 0.4888) – (mg K/d $\times$ 0.0205) − (mg Ca/d $\times$ 0.0125) − (mg Mg/d $\times$ 0.0263) (22). The values of dietary phytate and minerals were analytically measured. Based on the participants' average energy intake of 9.4 MJ (2250 kcal)/d, the LPLP and HPHP diets contained ~61 and 118 g protein/d or ~0.8 and 1.6 g protein/kg body weight, respectively. Differences in protein contents were achieved primarily by increasing portions of meat protein (mainly from beef) resulting in 12 and 68 g meat protein for the LPLP and HPHP diets, respectively. To maximize the PRAL difference, compared with the LPLP diet, the isocaloric HPHP diet not only had a higher meat content but also contained more grain products and fewer potassium-rich vegetables and fruits. Accordingly, the LPLP diet emphasized potato products and plenty of fruits, vegetables, and nuts, whereas the HPHP diet provided rice, pasta, and other grain products and limited amounts of fruits and vegetables. As a result, the LPLP and HPHP diets had ~−48 and 33 mEq/d PRAL, respectively, for a difference in PRAL values between 2 diets of ~81 mEq/d.

TABLE 1 Baseline characteristics of the study participants[1]

Characteristics	
Age, *y*	56.9 ± 3.2
Weight, *kg*	71.4 ± 10.1
BMI	26.8 ± 3.1
Serum	
Ionized Ca, *mmol/L*	1.2 ± 0.1
TRAP, *U/L*	5.1 ± 0.5
Creatinine, *μmol/L*	64.2 ± 10.6
C-telopeptide of type I collagen, *pmol/L*	10 ± 5
Intact PTH, *pmol/L*	7.1 ± 2.0
OC, *nmol/L*	1.7 ± 1.0
IGF-I, *nmol/L*	16.6 ± 5.9
OPG, *pmol/L*	5.5 ± 0.6
sRANKL, *pmol/L*	286 ± 174
25-hydroxycholecalciferol, *nmol/L*	60.5 ± 16.3
Urine[2]	
Ca, *mmol/d*	4.0 ± 2.1
Mg, *mmol/d*	2.9 ± 1.0
P, *mmol/d*	22 ± 7
K, *mmol/d*	43 ± 16
Na, *mmol/d*	114 ± 37
Creatinine, *mmol/d*	5.3 ± 3.2
Oxalate, *mmol/d*	0.32 ± 0.29
pH	6.2 ± 0.4
Ammonium, *nmol/d*	28 ± 7
Titratable acidity, *mEq/d*	20 ± 11
Free organic acid, *mEq/d*	32 ± 6
N-telopeptide, *nmol BCE/d*	201 ± 87
N-telopeptide, *nmol BCE/mmol creatinine*	44 ± 23
DPD,[3] *nmol/L*	7.5 ± 2.6

[1] Values are mean ± SD, n = 16.
[2] Urine data are for 24-h urine collection unless indicated otherwise.
[3] First morning void.

Skim milk was the primary source of calcium for both diets (Table 2). The amounts of milk were divided into equal portions at each meal. The study was conducted in Grand Forks (~48° N), North Dakota, between late January and early May. To stabilize body vitamin D status, each participant received a daily supplement of 10 μg cholecalciferol starting 3 wk before the controlled diets began and continuing through the rest of the study. Beginning at the start of the diet, all participants received an additional daily multivitamin tablet containing 10 μg cholecalciferol (Table 2, footnote). The skim milk was also fortified with vitamin D, providing an additional 2.5 μg to a 9.4-MJ diet.

To maintain body weights, energy intakes were adjusted by proportionally changing the amounts of all foods. Coffee; tea; artificially sweetened, noncola carbonated beverages (containing citric acid rather than phosphoric acid); and salt intakes were individualized, limited to 2 total servings/d and kept constant during the study. The consumption of city water and chewing gum was not controlled after analyses indicated minimal mineral content. The women were given a list of approved over-the counter medications, toothpaste, and dental adhesives that contained minimal amounts of calcium or other minerals. All diet ingredients except water were weighed to 1% accuracy and, as possible, were purchased from single production lots. Participants consumed the food quantitatively, with the aid of spatulas and rinse bottles, consuming 1 meal (breakfast) at the Research Center on weekdays and the remaining foods elsewhere.

Ca absorption measurements with ^{47}Ca. After 3 wk of equilibration to each diet, all the meals of the 2-d menu were extrinsically labeled with a total of 148 kBq (4 μCi) ^{47}Ca radiotracer (the activity as of midnight between the 2 d). The isotope (with a radioactive half-life of 4.5 d) was obtained by neutron activation (University of Missouri, Columbia, MO) of stable ^{46}Ca (as Ca bicarbonate, 30.89% enriched; Oak Ridge

TABLE 2 Nutrient composition and PRAL for experimental diets[1–3]

	LPLP	HPHP
Protein, % energy	10	20
Total protein, *g*	61	118
Meat protein, *g*	12	68
Protein/body weight, *g/kg*	0.86 ± 0.08	1.70 ± 0.21
Fat, *% energy*	30	30
Carbohydrate, *% energy*	60	50
Dietary fiber, *g*	28	20
Dietary phytate, *mg*	661 ± 11	790 ± 3
Calcium, *mg*	907 ± 55	865 ± 96
Phosphorus, *g*	1.27 ± 0.22	1.79 ± 0.06
Magnesium, *mg*	387 ± 10	334 ± 16
Potassium, *g*	5.00 ± 0.11	3.45 ± 0.12
Sodium, *g*	3.81 ± 0.33	3.56 ± 90.5
PRAL, *mEq*[4]	−48	33

[1] Data are mean ± SD, $n = 3$ for the daily composition of the 2-d menus, based on the participants' mean energy intake of 9.4 MJ/d.
[2] Protein, fat, carbohydrate, and dietary fiber values were calculated based on USDA nutrient composition data (26). Phytate, mineral, and electrolyte intakes were analyzed in triplicate as described in the text.
[3] Supplemented with a daily vitamin D supplement containing 10 μg cholecalciferol and a daily multivitamin tablet containing vitamin A acetate (1391 μg), ascorbic acid (170 mg), cholecalciferol (10 μg), dl-α-tocopherol acetate (13 mg), pyridoxine (4.1 mg), cyanocobalamin (13.4 μg), thiamin (3.4 mg), riboflavin (4.0 mg), niacin (50 mg), and folic acid (1172 μg).
[4] PRAL was calculated (22) as: PRAL (mEq/d) = (mg P/d × 0.0366) + (g protein/d × 0.4888) − (mg K/d × 0.0205) − (mg Ca/d × 0.0125) − (mg Mg/d × 0.0263).

National Research Laboratory, Oak Ridge, TN). Calcium-47 was added to milk in proportion to the Ca content of each meal so that the specific activity of the isotope was constant for each meal. The labeled milk was allowed to equilibrate for at least 12 h before administration. All labeled meals were consumed under supervision at the research center.

Ca retention from the diet was determined with a custom-made scintillation counter (27,28), which detects gamma emissions with 32 crystal NaI(Tl) detectors (10 × 10 × 41 cm each) arranged in 2 planes above and below a bed on which the participants lie. Whole body scintillation counting was performed before (background) and 1–3 h after the first labeled meal (before any isotope was excreted), then twice each week for the remainder of each dietary period. Whole body counting data were corrected for radioactive decay to the midpoint of the days of labeled meals. The precision of the whole body counting measurements was 1.4%. The measured ^{47}Ca retention is presented for d 21. In addition, the fractional Ca absorption was estimated from the y-axis intercept of the linear portion (d 13–28 for most volunteers, d 16–28 for 1 volunteer with an apparently longer gastrointestinal transit time) of a semilogarithmic retention plot of percent ^{47}Ca retained compared with time (29).

Biochemical measurements. Blood samples were drawn after an overnight fast at wk 0 (prior to starting the diet), 3, 5, and 7 of each dietary period. The women provided 2 consecutive 24-h urine samples during wk 0, 1, 2, 3, 5, and 7 of each dietary period to monitor renal acid and Ca excretions and any adaptive response to the dietary treatments. To minimize variability, samples were held and measured in the same analytical batch for each volunteer for those clinical measurements that are known to remain stable when frozen.

Dietary phytate was determined by acid extraction, ion exchange separation, and phosphorus analysis and quantified by assuming 6 mol phosphorus/mol phytic acid (30).

Plasma ionized Ca was measured with an electrode (Nova-CRT 8 Analyzer) (31). Urine was acid-diluted and diet was acid-digested for measurement of minerals by inductively coupled argon plasma emission spectrophotometry. Analytical quality was monitored by analyzing standard reference materials (Seronorm Trace Elements Urine, Lot 2525, SERO; Typical Diet, 1548b, U.S. National Institute of Standards and Technology).

Serum tartrate-resistant acid phosphatase (TRAP) activity was determined using α-nanphthyl-phosphate and diazotized-2-amino-5-chlorotoluene as substrates using the commercial kit (no. 200737321) (Cobas Integra, Roche Diagnostic) (32). Serum and urinary creatinine were measured with buffered kinetic Jaffe reaction without deproteinization (Cobas Integra, Roche Diagnostic) (33). Serum carboxyterminal cross-linking telopeptide (CTX) of bone collagen concentration was measured using an ELISA kit (no. 4CRL4000, Nordic Bioscience Diagnostics). Commercial kits were used to measure serum concentrations of intact parathyroid hormone (PTH; kit no. LPP1), osteocalcin (OC; kit # LKON1), IGF-1 (kit no. LKGF1), and deoxypyridinoline (DPD; kit no. LKPD1) using a solid phase chemiluminescent ELISA with an automated immunoassay system (Immulite 1000; Diagnostic Products). Serum 25-hydroxycholecalciferol concentration was measured using 25-hydroxycholecalciferol pre-extraction with acetonitrile and assayed using double antibody RIA (DiaSorin).

Serum osteoprotegerin (OPG; no. RD194003200) and soluble receptor activator nuclear factor-κB ligand (sRANKL; no. RD198014200R) were measured using ELISA kits from Biovendor. Urinary aminoterminal cross-linking telopeptide of bone collagen (NTX) was measured with Osteomark NTX ELISA kit from Wampole Laboratories.

Urine oxalate was measured colorimetrically (Oxalate kit no. 591-D, Trinity Biotech). Urinary ammonium was measured colorimetrically (Sigma Aldrich). Titratable acidity was determined in undiluted urine by titrating to pH 7.40 with 0.1 mol/L NaOH. Urinary free organic acids were measured by the method of Remer and Manz (24).

Statistics. Effects of diet and treatment sequence on blood and urine variables were analyzed by using repeated-measures ANCOVA with the corresponding baseline value of each variable as a covariate (34). The effects of diets and treatment sequence on Ca absorption were evaluated using mixed model ANOVA. All ANOVA and ANCOVA were run using the Mixed procedure in SAS version 9.2 (SAS Institute). Tukey's contrasts were used for post hoc comparisons. Treatment sequence was not significant and thus not reported. Variances in the data are reported as the pooled SD for the diet × time means from the ANCOVA and for the diet means from the ANOVA. Some data (as indicated in the tables) were logarithmically transformed prior to analysis, because either they were not normally distributed or the variances were not homogeneous. Using 2-tailed probabilities, $P \leq 0.05$ was considered significant. Values in the text are means and pooled SD.

Results

Ca absorption and excretion. Compared with the LPLP diet, the HPHP diet significantly increased the fraction of the Ca isotope retained by the body at 21 d after isotope administration (**Table 3**). Compared with the LPLP diet, the HPHP diet increased

TABLE 3 Ca retention and absorption in postmenopausal women consuming controlled LPLP and HPHP diets for 7 wk each in a crossover design[1]

	LPLP	HPHP	Pooled SD	*P*-value
^{47}Ca retention at d 21,[2] %	16.9	19.7	3.8	0.05
Absorption,[3] %	22.3	26.5	5.4	0.05
Ca absorbed, *mg/d*	200	227	46	0.12

[1] Values are least square means and pooled SD, $n = 16$.
[2] Retention of Ca tracer at d 21 corrected for isotopic decay and expressed as percent of the oral dose.
[3] Intestinal Ca absorption, as percent of dose, was estimated by using the linear portion (d 13–28 after ^{47}Ca administration for most volunteers, d 16–28 for 1 volunteer with an apparently longer gastrointestinal transit time) of a semilogarithmic retention plot [ln (% retention vs. time)] and extrapolating back to the time of tracer administration.

the percent of Ca absorbed from the diet ($P < 0.05$) (Table 3). Despite a slightly greater Ca content of the LPLP diet (Table 2), this increased efficiency of Ca absorption tended to increase the amount of Ca absorbed from the HPHP diet ($P < 0.12$) (Table 3).

The higher level of dietary protein increased urinary Ca excretion ($P = 0.005$) for the 7-wk diet period (156 vs. 203 ± 63 mg/d for the LPLP and the HPHP diets, respectively). The diet-related difference in urinary Ca excretion was consistent from wk 1 through 7, with no indication of adaptation [the effect of time and the interaction between diet and time were not significant ($P > 0.4$)] (**Table 4**). Assuming that Ca absorption remained the same throughout the study, the net difference between Ca absorbed (Table 3) and urinary excretion (Table 4) did not differ between the LPLP and HPHP diet periods (55 vs. 28 ± 51 mg/d for the LPLP and the HPHP diets, respectively, $P > 0.05$).

Urinary magnesium was lower and phosphorus was higher when the women consumed the HPHP diet compared with the LPLP diet, reflecting dietary mineral content (Table 4). The dietary treatments did not affect urinary chloride and sodium excretions (Table 4).

TABLE 4 Urine excretions and concentrations in healthy postmenopausal women consuming controlled LPLP and HPHP diets for 7 wk each in a crossover design[1]

								ANCOVA *P*-values		
	wk 0[2]	wk 1	wk 2	wk 3	wk 5	wk 7	Pooled SD	Diet	Week	Diet × week
Calcium, *mmol/d*							1.7	0.005	0.60	0.43
LPLP	4.2	3.9	3.6	3.9	4.2	4.0				
HPHP	5.3	5.0	5.2	4.9	5.3	5.0				
Magnesium, *mmol/d*							0.9	0.03	0.27	0.30
LPLP	2.9	4.1	3.8	3.8	4.0	3.9				
HPHP	3.6	3.3	3.5	3.1	3.5	3.6				
Phosphorus, *mmol/d*							5	<0.0001	0.02	0.05
LPLP	23	19	17	17	19	17				
HPHP	26	30	30	27	30	32				
Potassium, *mmol/d*							13	<0.0001	0.42	0.09
LPLP	52	85	79	85	82	85				
HPHP	57	54	59	51	56	62				
Chloride, *mmol/d*							30	0.16	0.28	0.33
LPLP	82	88	76	82	79	84				
HPHP	86	77	74	70	71	68				
Sodium, *mmol/d*							26	0.99	0.22	0.01
LPLP	139	139	113	134	139	139				
HPHP	147	130	135	122	135	139				
Creatinine, *mmol/d*							1.1	<0.0001	0.26	0.19
LPLP	5.3	3.6	4.0	3.9	3.9	3.9				
HPHP	5.1	5.1	4.8	5.0	5.7	5.6				
Ln (oxalate),[3] *mmol/d*							0.36	0.0004	0.36	0.96
LPLP	−1.47 (0.23)	−1.14 (0.32)	−1.20 (0.30)	−1.18 (0.31)	−1.24 (0.29)	−1.20 (0.30)				
HPHP	−1.31 (0.27)	−1.38 (0.25)	−1.52 (0.22)	−1.45 (0.23)	−1.56 (0.21)	−1.48 (0.23)				
pH							0.2	<0.0001	0.23	0.71
LPLP	6.2	7.1	7.0	7.1	7.1	7.1				
HPHP	6.2	5.9	5.9	6.0	6.0	5.9				
Ammonium, *mmol/d*							5.7	<0.0001	0.14	0.07
LPLP	27	13.7	14.7	14.5	13.8	13.2				
HPHP	28	39.9	38.9	35.4	34.1	38.3				
Titratable acidity, *mEq/d*							4.6	<0.0001	0.03	0.27
LPLP	18.2	3.9	4.2	3.2	3.1	3.6				
HPHP	17.9	25.6	23.4	20.8	21.9	22.6				
Ln (Free organic acid),[3] *mEq/d*							0.24	<0.0001	0.91	0.87
LPLP	3.4 (28)	3.7 (41)	3.7 (39)	3.7 (42)	3.7 (39)	3.7 (40)				
HPHP	3.5 (32)	3.3 (26)	3.3 (26)	3.3 (26)	3.3 (26)	3.2 (25)				
NTX, *nmol bone collagen equivalent/d*							153	0.41	0.22	0.71
LPLP	208	176	198	198	194	227				
HPHP	179	218	202	206	238	270				
Ln (DPD),[3,4] *nmol/L*							0.61	0.20	0.10	0.36
LPLP	3.8 (45)	3.5 (31)	3.7 (42)	3.4 (29)	3.6 (37)	3.5 (32)				
HPHP	3.7 (40)	3.6 (35)	3.6 (36)	3.7 (39)	3.9 (50)	3.7 (41)				

[1] Values are least square means from the ANCOVA with pooled SD for 24-h urine unless otherwise indicated, $n = 16$.
[2] Values of each variable at wk 0 are used as covariates.
[3] For logarithmically transformed data, the geometric mean in the untransformed units is indicated in brackets.
[4] Values are for the first morning void.

TABLE 5 Serum biochemistry in healthy postmenopausal women consuming controlled LPLP and HPHP diets for 7 wk each in a crossover design[1]

						ANCOVA *P*-values		
	wk 0[2]	wk 3	wk 5	wk 7	Pooled SD	Diet	Week	Diet × week
Ionic Ca, *mmol/L*					0.003	0.11	0.16	0.72
LPLP	1.20	1.19	1.20	1.21				
HPHP	1.19	1.21	1.21	1.22				
TRAP, *U/L*					0.49	0.44	0.30	0.90
LPLP	5.46	4.83	4.95	5.07				
HPHP	5.21	4.95	5.07	5.09				
Creatinine, *μmol/L*					6	0.82	0.0005	0.44
LPLP	63.9	71	65	66				
HPHP	63.9	70	67	66				
Ln (CTX),[3] *pmol/L*					0.25	0.87	0.001	0.58
LPLP	2.11 (8.3)	2.15 (8.6)	2.05 (7.8)	2.09 (8.1)				
HPHP	2.09 (8.1)	2.21 (9.1)	2.05 (7.8)	2.04 (7.7)				
Ln (intact PTH),[3] *pmol/L*					0.25	0.001	0.34	0.06
LPLP	1.8 (5.8)	1.8 (6.0)	1.8 (6.3)	1.8 (6.2)				
HPHP	1.8 (6.0)	1.7 (5.6)	1.6 (4.7)	1.6 (4.9)				
Ln (OC),[3] *nmol/L*					0.44	0.88	0.99	0.41
LPLP	0.27 (1.31)	0.14 (1.16)	0.28 (1.33)	0.26 (1.30)				
HPHP	0.18 (1.20)	0.32 (1.37)	0.20 (1.22)	0.22 (1.24)				
Ln (IGF-I),[3] *nmol/L*					0.22	0.0001	0.32	0.55
LPLP	2.85 (17.4)	2.87 (17.7)	2.90 (18.2)	2.89 (18.0)				
HPHP	2.85 (17.4)	3.09 (21.9)	3.12 (22.7)	3.20 (24.5)				
OPG, *pmol/L*					0.53	0.38	<0.0001	0.87
LPLP	5.41	5.13	5.17	4.72				
HPHP	5.45	5.00	5.12	4.58				
Ln (sRANKL),[3] *pmol/L*					0.35	0.25	0.04	0.68
LPLP	5.0 (148)	5.0 (153)	5.0 (142)	4.9 (137)				
HPHP	5.14 (171)	5.0 (152)	4.8 (125)	4.8 (118)				
25(OH)VitD, *nmol/L*					13	0.76	0.24	0.97
LPLP	60	62	65	64				
HPHP	61	61	65	63				

[1] Values are least square means with pooled SD from the ANCOVA, $n = 16$.
[2] Values of each parameter at wk 0 are used as covariates.
[3] For logarithmically transformed data, the geometric mean in the untransformed units is indicated in brackets.

Other biochemical measurements. The HPHP diet significantly increased urinary acidity (Table 4) compared with the LPLP diet. Women had higher urinary ammonium ion excretions and titratable acidity when they consumed the HPHP diet than when they consumed the LPLP diet ($P < 0.0001$). As a result, the urinary pH differed by 1.2 units during the 2 diet periods ($P < 0.0001$). The differences in ammonium ions and urinary pH were consistent from wk 1 through 7, suggesting that urinary acid excretion responded to diet treatments within the first week without further adaptation (Table 4). The HPHP diet increased urinary creatinine excretion (Table 4) compared with the LPLP diet ($P < 0.0001$), reflective of the greater dietary meat content. The dietary treatments had no significant effects on urinary bone resorption markers, either NTX in 24-h urine or DPD in the first morning void. The HPLP diet reduced urinary excretion of free organic acids ($P < 0.0001$) and oxalate ($P = 0.0004$) compared with the LPLP diet, likely reflecting the greater fruit and vegetable content of the LPLP diet.

Compared with the LPLP diet, the HPHP diet increased serum IGF-I concentrations ($P < 0.0001$) and decreased serum intact PTH concentrations ($P < 0.001$) (**Table 5**). The dietary treatments did not significantly affect other serum biomarkers, such as ionic Ca, TRAP, creatinine, CTX, OC, OPG, and sRANKL.

The participants were provided with vitamin D supplements prior to and during the study to prevent possible changes in vitamin D status that had the potential to confound the Ca absorption measurements. Based on measurements of serum 25-hydroxy cholecalciferol at wk 3, 5, and 7 of each diet period, the vitamin D status of the participants did not change during the study. For the resulting range of serum 25-hydroxycholecalciferol concentrations (32.5–95 nmol/L), there was no relationship between this variable and Ca absorption ($P > 0.05$) as evaluated separately for each of the 2 dietary treatments.

Discussion

In this 15-wk controlled study, we have shown that compared with a diet with low meat protein and low PRAL, a diet with high meat protein and high PRAL increased the fractional absorption of dietary Ca, which nearly compensated for the increased urinary Ca excretion.

The amount of dietary Ca absorbed was greater than urinary Ca excretion for both diets; this was expected, because the dietary Ca contents in this study are commonly associated with positive Ca balance (35). Relative to the LPLP diet, the HPHP diet increased urinary Ca excretion, a finding consistent with

other reports (18,20,36–38). Compared with the LPLP diet, the HPHP diet contained 57 g/d more protein and increased urinary Ca excretion by 47 mg/d, an amount similar to findings by other investigators (18,39). In a study with similar dietary protein contents to those of the current study but a smaller PRAL difference between the 2 diets (20), the high-protein diet increased urinary Ca excretion by only 23 mg/d. This is consistent with the proposal that dietary acid load rather than protein is the main contributing factor in increasing urinary Ca excretion. Counteracting acidosis with base-forming minerals has been shown to decrease the urinary Ca excretion attributed to high-protein intake (40–43). Our data on Ca absorption and urinary Ca excretion should not be used as an indicator of whole body Ca balance or Ca mobilization from bone without measuring fecal and/or endogenous Ca excretion. For example, Kerstetter et al. (18) reported that although a high-protein diet increased urinary Ca excretion, it actually decreased the fraction of urinary Ca of bone origin. However, the increased fractional Ca absorption from the HPHP diet may have at least partially compensated for the increase in urinary Ca excretion.

The amounts of Ca in our diets were slightly below the DRI of 1000 mg Ca/d (44) but higher than typical intakes by postmenopausal women in the United States (44) or Ca levels in similar studies investigating protein effects on Ca metabolism (17,18,20). The 4 percentage point increase in fractional Ca absorption when women consumed the HPHP compared with the LPLP diet containing ~900 mg/d Ca (Table 3) in the present study is comparable to another study where diets contained ~800 mg/d (18). However, dietary protein's enhancement of Ca absorption may depend on dietary Ca content; in 1 study (20), dietary protein significantly increased Ca absorption if the diet provided 675 mg Ca/d, but not if it provided 1510 mg Ca/d. Protein may increase Ca absorption by increasing its solubility in the intestinal lumen, whereas high Ca intakes may saturate Ca absorption receptors, limiting the enhancing effect of protein (18).

As expected from the diet composition, urinary titratable acidity was increased by the HPHP diet, whereas pH was decreased, a result consistent with other studies (17,20). Although previous results suggested a possible adaptation in urinary acidity with time (17), no adaptation in urinary acidity or Ca excretion was observed within 7 wk in the present study.

IGF-I is a potent anabolic agent that increases bone formation and bone mass through increasing osteoprogenitor number and proliferation (45). In the present study, the HPHP diet increased serum IGF-I by >20% compared with the LPLP diet, a finding confirmed by many other studies (20,46–49). Similar to some (50,51) but not all reports (17,20), the serum concentrations of PTH were significantly lower when women consumed the HPHP diet compared with the LPLP diet. In the present and other studies (50,51), the decrease in serum PTH may have been a normal compensatory response to the protein-associated increase in Ca absorption to maintain normal blood Ca levels.

Because each participant was provided with daily vitamin D supplements in addition to vitamin D-containing multivitamin tablets and vitamin D-fortified skim milk, the vitamin D status of the women in our study was within the normal range (52) using serum 25-hydroxycholecalciferol concentration as the indicator. Therefore, vitamin D status should not be a factor affecting our findings regarding Ca absorption data, because there were no differences in vitamin D status between the 2 groups.

The present study detected no change in potential biomarkers of osteoclast activity, such as blood TRAP, CTX, and sRANKL and urinary DPD, or biomarkers of osteoblast activity, such as blood OPG and OC. The observed changes in IGF-I and PTH were apparently insufficient to elicit detectable changes in biomarkers of osteoclast or osteoblast activity.

Many epidemiological observations have shown that long-term protein intakes are positively associated with bone mineral density (9,11,13,53). Several recent meta-analyses have concluded that protein is beneficial to bone health (54) and protein-induced acid load does not promote skeletal bone mineral loss or contribute to the development of osteoporosis (55,56). The results from this study are in agreement with those findings.

In conclusion, in postmenopausal women, a diet high in both protein and PRAL increased Ca absorption, at least partially compensating for an increase in urinary excretion. No change in either bone resorption or formation biomarkers was observed, indicating that a high-protein diet is not detrimental. However, the increased serum IGF-I combined with decreased serum PTH concentrations suggest that a high-protein diet could be beneficial to bone health.

Acknowledgments

We thank Dr. Steve Morris of Missouri Research Reactor for processing the radiotracer and Dr. Glenn Lykken for consulting on health physics and use of the whole body counter. J.J.C. initiated, designed, and supervised implementation of the study, helped analyze the results, and wrote the manuscript; L.K.J. provided statistical and computing expertise for planning and data analysis and reviewed the manuscript; and J.R.H. helped on study design, supervised implementation of the study, provided consultation on isotopic measurements, helped analyze the results, and revised the manuscript. All authors read and approved the final manuscript.

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