Calcium homeostasis and bone metabolic responses to high-protein diets during energy deficit in healthy young adults: a randomized controlled trial\(^1-4\)

Jay J Cao, Stefan M Pasiakos, Lee M Margolis, Edward R Sauter, Leah D Whigham, James P McClung, Andrew J Young, and Gerald F Combs Jr

ABSTRACT

**Background:** Although consuming dietary protein above current recommendations during energy deficit (ED) preserves lean body mass, concerns have been raised regarding the effects of high-protein diets on bone health.

**Objective:** The objective was to determine whether calcium homeostasis and bone turnover are affected by high-protein diets during weight maintenance (WM) and ED.

**Design:** In a randomized, parallel-design, controlled trial of 32 men and 7 women, volunteers were assigned diets providing protein at 0.8 [Recommended Dietary Allowance (RDA)], 1.6 (2 \times RDA), or 2.4 (3 \times RDA) g \cdot kg\(^{-1}\) \cdot d\(^{-1}\) for 31 d. Ten days of WM preceded 21 d of ED, during which total daily ED was 40\%, achieved by reduced dietary energy intake (\sim 30\%) and increased physical activity (\sim 10\%). The macronutrient composition (protein g \cdot kg\(^{-1}\) \cdot d\(^{-1}\) and % fat) was held constant from WM to ED. Calcium absorption (ratio of \(^{40}\)Ca to \(^{42}\)Ca) and circulating indexes of bone turnover were determined at day 8 (WM) and day 29 (ED).

**Results:** Regardless of energy state, mean (±SEM) urinary pH was lower (P < 0.05) at 2 \times RDA (6.28 ± 0.05) and 3 \times RDA (6.23 ± 0.06) than at the RDA (6.54 ± 0.06). However, protein had no effect on either urinary calcium excretion (P > 0.05) or the amount of calcium retained (P > 0.05). ED decreased serum insulin-like growth factor I concentrations and increased serum tartrate-resistant acid phosphatase and 25-hydroxyvitamin D concentrations (P < 0.01). Remaining markers of bone turnover and whole-body bone mineral density and content were not affected by either the protein level or ED (P > 0.05).

**Conclusion:** These data demonstrate that short-term consumption of high-protein diets does not disrupt calcium homeostasis and is not detrimental to skeletal integrity. This trial was registered at www.clinicaltrials.gov as NCT01292395.

INTRODUCTION

Energy deficit (ED),\(^5\), from either reduced dietary energy intake or increased energy expenditure, is used to induce weight loss by overweight and obese individuals. Healthy, normal-weight individuals such as athletes or military personnel who engage in intense physical activity or sustained military operations may also experience periods of ED. Although ED-induced weight loss results in diminished levels of fat mass, it also causes skeletal muscle and bone loss (1–4). It is estimated that up to 2% bone loss may occur at various bone sites consequent to a 10% weight loss in overweight/obese individuals (1). Reductions in muscle and bone mass may compromise physical performance, impede further weight loss, and increase the risk of falls, fractures, and injury.

Consumption of high-protein diets in excess of the Recommended Dietary Allowance (RDA; 0.8 g \cdot kg\(^{-1}\) \cdot d\(^{-1}\)—one of the accepted diet strategies for weight loss (5–8)—improves blood lipids and attenuates muscle loss (6, 7, 9, 10) during weight reduction. However, high-protein diets have been considered to be detrimental to bone health (11–14) because of the increase in urinary calcium excretion resulting from the metabolic acidity of protein catabolism (15–18). In contrast, several well-controlled human trials have shown that consumption of a high-protein diet can offset the increase in urinary calcium excretion by enhancing intestinal calcium absorption (19–23).

Limited evidence suggests that high-protein diets may attenuate total and trabecular bone loss in obese postmenopausal

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5. Abbreviations used: BAP, bone-specific alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; ED, energy deficit; IGF-I, insulin-like growth factor I; NTX, amino-terminal cross-linking telopeptide of bone collagen; PRAL, potential renal acid load; PTH, parathyroid hormone; RDA, Recommended Dietary Allowance; TRAP, tartrate-resistant acid phosphatase; V\(\text{O}_{2\text{peak}}\), peak oxygen uptake; WM, weight maintenance; 25(OH)D, 25-hydroxyvitamin D.

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women during weight loss (24). Whether high-protein diets alter
calcium absorption/retention during ED has not previously been
addressed. This study was part of a larger randomized controlled
trial designed to assess the musculoskeletal responses to habitual
consumption of dietary protein at levels exceeding the RDA
during short-term ED. We previously showed that consuming
dietary protein at levels above the RDA protects fat-free mass and
spares muscle protein anabolic sensitivity to dietary protein
during short-term weight loss (25). The objective of the current
study was to determine whether high-protein diets influence
spares muscle protein anabolic sensitivity to dietary protein
during short-term ED. We previously showed that consuming
consumption of dietary protein at levels exceeding the RDA
trial designed to assess the musculoskeletal responses to habitual
weight maintenance (WM) and ED. We hypothesized that ED
would diminish bone formation and increase bone resorption
with concomitant increases in urinary calcium excretion. How-
ever, we expected that consuming protein at levels above the
RDA would attenuate the effects of ED on markers of bone
metabolism and enhance calcium absorption, despite dietary
protein-induced increases in urinary calcium excretion.

SUBJECTS AND METHODS

Experimental design

The study was approved by the Human Subjects Committee of
the University of North Dakota and the Human Use Review
Committee of the US Army Research Institute of Environmental
Medicine. The protocol was explained verbally and in writing by
the investigators, and written informed consent was obtained
from each volunteer. Subjects were cleared as being in good
general health by the study physician and were admitted to the
metabolic ward at the Grand Forks Human Nutrition Research
Center for the duration of the study to ensure study compliance.

The study consisted of a randomized block design according
to sex, BMI, fitness level (peak oxygen uptake \( \dot{V}O_{2\text{peak}} \)), and
prestudy dietary intake. Volunteers were assigned to diets pro-
viding protein at 0.8 (RDA), 1.6 (2 × RDA), and 2.4 (3 × RDA)
g · kg\(^{-1}\) · d\(^{-1}\) for 31 d. A 10-d WM period was immediately
followed by 21 d of ED (40% ED = 30% dietary restriction +
10% increase in physical activity–induced energy expenditure).
Baseline characteristics such as age, height, weight, BMI, \( \dot{V}O_{2\text{peak}} \), and bone-related markers in blood and urine of the
study subjects are shown in Table 1.

Extended details regarding participants’ eligibility and
physical activity intervention were described previously (25).
The highly controlled individualized 3-d WM and ED menus
were prepared by research dietitians (see Supplemental Table 1
under “Supplemental data” in the online issue) and consumed in
a metabolic kitchen under supervision to ensure compliance.
Energy and macronutrient intakes were recorded and analyzed
(Table 2). To isolate the effects of dietary protein, and to limit

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RDA</th>
<th>2 × RDA</th>
<th>3 × RDA</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 2</td>
<td>176 ± 2</td>
<td>176 ± 2</td>
<td>—</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ± 2</td>
<td>76 ± 3</td>
<td>77 ± 2</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>( \dot{V}O_{2\text{peak}} ) (mL · kg(^{-1}) · min(^{-1}))</td>
<td>47 ± 1</td>
<td>48 ± 2</td>
<td>49 ± 2</td>
<td>—</td>
</tr>
<tr>
<td>Bone mineral density (g/cm(^2))</td>
<td>1.30 ± 0.03</td>
<td>1.22 ± 0.03</td>
<td>1.19 ± 0.02</td>
<td>0.112</td>
</tr>
<tr>
<td>Bone mineral content (kg)</td>
<td>3.04 ± 0.14</td>
<td>2.75 ± 0.12</td>
<td>3.00 ± 0.11</td>
<td>0.206</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase (U/L)</td>
<td>3.7 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>0.50</td>
</tr>
<tr>
<td>Creatinine (( \mu )mol/L)</td>
<td>86.7 ± 2.4</td>
<td>77.1 ± 3.1</td>
<td>79.6 ± 3.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Intact parathyroid hormone (pmol/L)</td>
<td>3.1 ± 0.5</td>
<td>3.7 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Insulin-like growth factor I (nmol/L)</td>
<td>29.5 ± 2.2</td>
<td>25.3 ± 2.3</td>
<td>27.4 ± 2.5</td>
<td>0.44</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase (U/L)</td>
<td>35.9 ± 6.3</td>
<td>31.8 ± 3.0</td>
<td>35.1 ± 3.7</td>
<td>0.84</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D (nmol/L)</td>
<td>69.8 ± 6.0</td>
<td>57.3 ± 3.5</td>
<td>65.3 ± 5.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>202.0 ± 35.0</td>
<td>164.8 ± 25.5</td>
<td>172.6 ± 22.6</td>
<td>0.620</td>
</tr>
<tr>
<td>Potassium (g/d)</td>
<td>3.0 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>0.006</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>108.2 ± 10.1</td>
<td>72.6 ± 5.2</td>
<td>92.7 ± 7.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Sodium (g/d)</td>
<td>5.2 ± 0.7</td>
<td>3.4 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>0.066</td>
</tr>
<tr>
<td>Phosphorus (g/d)</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.094</td>
</tr>
<tr>
<td>Chlorine (mg/d)</td>
<td>205.4 ± 20.5</td>
<td>146.6 ± 18.2</td>
<td>171.3 ± 21.4</td>
<td>0.118</td>
</tr>
<tr>
<td>Sulfate (mg/d)</td>
<td>22.6 ± 3.3</td>
<td>17.4 ± 1.9</td>
<td>26.6 ± 2.8</td>
<td>0.065</td>
</tr>
<tr>
<td>pH</td>
<td>6.4 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>6.1 ± 0.2</td>
<td>0.282</td>
</tr>
<tr>
<td>Ammonium (nmol/d)</td>
<td>24.4 ± 4.0</td>
<td>24.7 ± 2.6</td>
<td>23.3 ± 2.7</td>
<td>0.924</td>
</tr>
<tr>
<td>Titratable acidity (mEq/d)</td>
<td>22.1 ± 3.5</td>
<td>18.7 ± 2.9</td>
<td>23.8 ± 3.7</td>
<td>0.551</td>
</tr>
<tr>
<td>Free organic acid (mEq/d)</td>
<td>48.3 ± 2.9</td>
<td>44.9 ± 4.2</td>
<td>44.6 ± 6.2</td>
<td>0.809</td>
</tr>
<tr>
<td>N-telopeptide (nmol BCE)</td>
<td>71.4 ± 9.5</td>
<td>64.4 ± 11.2</td>
<td>48.9 ± 5.5</td>
<td>0.272</td>
</tr>
<tr>
<td>Deoxypyridinoline (nmol/d)</td>
<td>5.0 ± 0.8</td>
<td>4.6 ± 0.4</td>
<td>4.3 ± 0.3</td>
<td>0.746</td>
</tr>
</tbody>
</table>

\(^{1}\)All values are means ± SEMs. BCE, bone collagen equivalents; RDA, Recommended Dietary Allowance; \( \dot{V}O_{2\text{peak}} \), peak oxygen uptake.

\(^{2}\)Block randomized according to sex, BMI, fitness level (\( \dot{V}O_{2\text{peak}} \)), and prestudy dietary intake. Homogeneity was
confirmed by using a 1-way ANOVA. However, sex, BMI, fitness level, and prestudy dietary protein intake were blocking
variables; hence, homogeneity was forced. Data on sex, BMI, and \( \dot{V}O_{2\text{peak}} \) were reported previously (25).
the confounding effects of varying calcium intakes between diets, calcium intake was similar between protein groups and was provided at levels consistent with the current RDA (≈1000 mg/d). An additional 162 mg supplemental calcium was consumed daily, regardless of protein group assignment, as volunteers consumed a multivitamin and mineral supplement (One A Day Maximum; Bayer HealthCare) daily to ensure that micronutrient requirements were met. Dietary potential renal acid load (PRAL) was calculated (27) as follows:

\[
\text{PRAL (mEq/d)} = (\text{mg P/d} \times 0.0366) + (\text{g protein/d} \times 0.4888) - (\text{mg K/d} \times 0.0205) - (\text{mg Ca/d} \times 0.0125) - (\text{mg Mg/d} \times 0.0263)
\]

**Calcium absorption measurements by using dual stable isotopes (\(^{44}\text{Ca}\) and \(^{42}\text{Ca}\))**

Fractional calcium absorption was the primary outcome of the study and was measured on days 8 and 29 of the WM and ED diet interventions, respectively, by using dual stable calcium isotopes (\(^{44}\text{Ca}\) and \(^{42}\text{Ca}\), as described by Ceglia et al (28). The stable isotopes were purchased from Trace Sciences International Inc and supplied as carbonate salt. The isotopic enrichments for \(^{44}\text{Ca}\) and \(^{42}\text{Ca}\) were >97% and >83%, respectively. Isotopes were prescribed by the study physician and prepared by the In-Health Specialty Pharmacy and certified sterile and pyrogen-free before use. After an overnight fast, a polyethylene catheter was inserted into an antecubital vein by a licensed medical laboratory technician for blood biochemical sampling and subsequent calcium stable isotope infusions, and volunteers were asked to empty their bladders. After breakfast, the volunteers were given \(^{44}\text{Ca}\) that had been premixed (0.375 and 0.575 mmol for volunteers <80 kg or ≥80 kg, respectively) with milk ≥12 h before administration. Immediately after the oral isotope administration, a 24-h urine collection began. Two hours after breakfast, \(^{42}\text{Ca}\) (0.0375 mmol for subjects <80 kg and 0.0575 mmol for those ≥80 kg) was infused intravenously over 2 min. The isotope concentrations in urine were measured by using inductively coupled plasma mass spectrometry, and the fractional calcium absorption was calculated as the ratio of urinary \(^{44}\text{Ca}\) to \(^{42}\text{Ca}\) as described previously (28, 29).

### TABLE 2

| Nutrient composition of WM- and ED-controlled diets |
|---------------------------------|----------------|----------------|----------------|----------------|
|                                 | RDA (n = 11, 2 F) | 2 × RDA (n = 11, 2 F) | 3 × RDA (n = 10, 2 F) |          |
|                                 | WM | ED | WM | ED | WM | ED |
| Energy                          |     |     |     |     |     |     |
| (kcal)                          | 2000 | 1400 | 2000 | 1400 | 2000 | 1400 |
| g protein                       |     |     |     |     |     |     |
| % of energy                     | 8   | 12  | 17  | 24  | 25  | 36  |
| (g)                             | 43  | 43  | 84  | 84  | 127 | 126 |
| (g/kg)                          | 0.8 | 0.8 | 1.6 | 1.6 | 2.4 | 2.4 |
| Fat (% of energy)               | 29  | 29  | 30  | 29  | 30  | 30  |
| Carbohydrate (% of energy)      | 62  | 59  | 54  | 48  | 45  | 35  |
| Dietary fiber (g)               | 18  | 14  | 19  | 14  | 18  | 11  |
| Calcium (mg)                    | 944 | 920 | 1004| 975 | 1017| 991 |
| Phosphorus (mg)                 | 1038| 1013| 1381| 1315| 1751| 1646|
| Magnesium (mg)                  | 315 | 290 | 355 | 316 | 392 | 349 |
| Potassium (mg)                  | 2397| 2127| 2667| 2447| 3183| 2988|
| Sodium (mg)                     | 2920| 2589| 3523| 3039| 4467| 3900|
| PRAL (mEq/d)                    | -8.97| -3.45| 16.46| 19.88| 38.99| 40.12|

1 Average 3-d WM and ED dietary intake for volunteers assigned to the RDA groups. Nutrient composition was derived from the USDA Nutrient Database for Standard Reference Release 24 (26). A One A Day Maximum (Bayer HealthCare) vitamin was consumed daily by each volunteer regardless of dietary protein group, which provided additional calcium (162 mg), phosphorus (109 mg), magnesium (100 mg), and potassium (80 mg). ED, energy deficit; PRAL, potential renal acid load; RDA, Recommended Dietary Allowance; WM, weight maintenance.

2 Calculated (27) 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).

**Measurements of bone biomarkers and bone mineral density and content**

Blood samples were drawn after an overnight fast. Timed 24-h urine samples were collected once or twice daily (after breakfast and/or immediately after the oral isotope administration) on 3 study days: days 0 (before initiating the WM diet intervention), 8, and 29.

Serum tartrate-resistant acid phosphatase (TRAP) activity was determined by using α-naphthyl-phosphate and diazotized-2-amino-5-chlorotoluene as substrates with a commercial kit (no. 200737321) (Cobas Integra; Roche Diagnostic Corp) (30). Serum creatinine was measured by using the buffered kinetic Jaffe reaction without deproteinization (Cobas Integra; Roche Diagnostic Corp) (31). Commercial kits were used to measure serum concentrations of intact parathyroid hormone (PTH; kit no. LPP1) and insulin-like growth factor I (IGF-I; kit no. LKGF1) by using a solid-phase chemiluminescent enzyme-labeled immunoassay with an automated immunoassay system (Immulite 1000; Diagnostic Products Corporation). Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured by using 25(OH)D preextraction with acetonitrile and assayed by using a double-antibody radioimmunoassay (DiaSorin Inc). Serum bone-specific alkaline phosphatase (BAP) was measured by using the MicroVue BAP EIA kit (Quidel Corp).

The total nitrogen content of the urine was measured by using pyrocheluminescence (Antek 9000; Antek Instruments), and partial results were presented again in this article to demonstrate compliance with the different levels of protein intake. Urinary aminoterminal cross-linking telopeptide of bone collagen (NTX) was measured by using the Osteomark NTX ELISA kit from Wampole Laboratories. Urine oxalate was measured colorimetrically (Oxalate Kit no. 91-D; Trinity Biotech), and ammonium was measured colorimetrically (Sigma Aldrich). Titratable acidity was determined in undiluted urine by titrating to pH 7.40 with 0.1 mol NaOH/L. Urinary free organic acids were measured by the method of Remer and Manz (32).

Urinary was acid-diluted, and research diets were acid-digested for measurement of minerals by inductively coupled argon plasma emission spectrophotometry. Analytic quality was monitored by analyzing standard reference materials (Seronorm Trace Elements Urine, Lot 2525, SERO AS, Typical Diet, 1548b, US National Institute of Standards and Technology).

Whole-body bone mineral content (BMC; g) and bone mineral density (BMD; g/cm²) were assessed by using dual-energy X-ray absorptiometry (Hologic Delphi-W, software version 11.2.1.7).

Within-day and between-day variabilities for BMD were 0.18%
Statistical analysis

Volunteers were block randomized by an independent statistician by sex (male and female), BMI (in kg/m²; 22–25 and 25.1–29), $\dot{V}O_{\text{peak}}$ (40–50 and 50.1–60 mL·kg$^{-1}$·min$^{-1}$), and pretest dietary protein intake (0.8–1.6 and 1.61–2.4 g·kg$^{-1}$·d$^{-1}$) by using Taves’ minimization method of treatment assignment (33). Power analyses (>85%) on the study primary outcome variable, muscle protein synthesis, indicated that 12 volunteers per group were necessary to detect differences in muscle protein synthesis (25). For fractional calcium absorption, 12 volunteers per group would provide 90% power to detect a mean difference of 0.11 between groups, assuming an SD of 0.07 and a mean difference of 0.11 between groups, assuming an SD of 12 volunteers per group would provide 90% power to detect a mean difference of 0.11 between groups, assuming an SD of 0.07 and $\alpha = 0.05$ (21).

Baseline volunteer characteristics are described by using common descriptive statistics and a 1-factor ANOVA confirmed homogeneity between diet groups. Effects of diet (RDA compared with 2 × RDA confirmed with 3 × RDA) and treatment phases (WM compared with ED) on blood and urine variables were analyzed by using a mixed-model ANOVA, in which diet was a between-subject factor and treatment phase was a within-subject factor. After the observation of a significant main or interactive effect, post hoc analyses were conducted by using Bonferroni adjustments. The $\alpha$ level for significance was set at $P < 0.05$. Data were analyzed by using Proc Glimmix in SAS, V9.3 (SAS Institute Inc) and are expressed as means ± SEMs.

RESULTS

As reported by Pasiakos et al (25), body weight remained stable during WM (overall mean ± SEM: 77.7 ± 1.5 kg) and was not significantly different ($P > 0.05$) between dietary protein groups. Total body mass loss was similar among dietary protein groups: 3.5 ± 0.3 kg for RDA, 2.7 ± 0.2 kg for 2 × RDA, and 3.3 ± 0.3 kg for 3 × RDA. The proportion of total body mass loss attributed to reductions in fat mass and fat-free mass differed by dietary protein groups, because the percentage of fat mass loss was higher ($P < 0.05$) and fat-free mass loss ($P < 0.05$) was lower for 2 × RDA (70.1 ± 7%, 29.8 ± 7%) and 3 × RDA (63.6 ± 5%, 36.4 ± 5%) than for RDA (41.8 ± 5%, 58.2 ± 5%). Changes in fat mass and fat-free mass were not significantly different ($P > 0.05$) between 2 × RDA and 3 × RDA.

There were no differences ($P > 0.05$) in fractional calcium absorption, whole-body BMD, and BMC between dietary protein levels (Table 3). Mean changes in fractional calcium absorption from WM to ED were similar between dietary protein groups ($P > 0.05$). ED had no effects on BMD and BMC ($P > 0.05$) but tended to increase fractional calcium absorption ($P = 0.055$). No protein × phase interactions were observed for fractional calcium absorption, BMD, and BMC.

<table>
<thead>
<tr>
<th>Protein Phase</th>
<th>Protein × Phase</th>
<th>RDA (n = 10 M, 2 F)</th>
<th>WM (n = 11 M, 2 F)</th>
<th>ED (n = 11 M, 2 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td></td>
<td>32.2 ± 0.6</td>
<td>33.2 ± 1.6</td>
<td>33.4 ± 1.6</td>
</tr>
<tr>
<td>WM × Phase</td>
<td></td>
<td>1.90 ± 0.03</td>
<td>1.31 ± 0.03</td>
<td>1.22 ± 0.03</td>
</tr>
<tr>
<td>ED</td>
<td></td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3: Fractional calcium absorption, whole-body bone mineral density, and whole-body bone mineral content during the 10-d WM and 21-d ED diets.
Overall, dietary protein had no effect on urinary calcium excretion ($P = 0.271$) (Table 4). On the basis of the estimated dietary calcium content (Table 2) and measures of fractional calcium absorption (Table 3), the amounts of calcium absorbed were 302 ± 14, 328 ± 11, and 343 ± 14 mg/d for RDA, 2 × RDA, and 3 × RDA, respectively, which did not differ among protein levels ($P > 0.05$). Calcium retention (the difference between the amount of calcium absorbed and excreted in urine) was not different between dietary protein levels (156 ± 24, 150 ± 25, and 132 ± 25 mg/d for RDA, 2 × RDA, and 3 × RDA, respectively) ($P > 0.05$). Differences were detected in urinary potassium ($P = 0.029$), sodium ($P = 0.018$), phosphorus ($P < 0.001$), and sulfate ($P < 0.001$) between dietary protein levels likely reflecting dietary mineral content, as shown in Table 2. ED decreased the urinary excretion of potassium ($P = 0.02$), sodium ($P = 0.009$), and chloride ($P = 0.008$). No protein × phase interactions were observed.

Consistent with study design, dietary protein levels increased urinary nitrogen ($P < 0.001$), ammonium ions ($P = 0.001$), and titratable acid ($P < 0.001$) (Table 4). Subjects who consumed 2 × RDA and 3 × RDA had higher urinary ammonium ions than did those who consumed the RDA diet ($P < 0.05$). As a result, urinary pH decreased with the increase in dietary protein levels ($P = 0.001$). Urinary pH values were lower ($P = 0.002$) during ED than during WM. ED had no effects on the urinary bone-resorption markers NTX and deoxypyridinoline ($P > 0.05$). No protein × phase interactions were observed for urinary variables ($P > 0.05$).

Dietary protein levels had no effects on serum bone biomarkers ($P > 0.05$), such as BAP, IGF-I, intact PTH, and TRAP (Table 5). ED decreased the circulating concentration of IGF-I ($P = 0.003$) by 14% and increased serum TRAP ($P = 0.008$) by 8%. Mean changes in serum IGF-I and TRAP from WM to ED were similar between the dietary protein groups ($P > 0.05$). ED increased serum 25(OH)D concentrations ($P = 0.001$). No protein × phase interactions were observed.

**DISCUSSION**

The major findings of this 31-d controlled study was that habitual consumption of dietary protein at levels above the RDA did not significantly alter urinary calcium excretion, dietary calcium retention, or markers of bone turnover or BMD, despite increased urinary acidity. These results indicate that diets that are 2 or 3 times the RDA for protein are not detrimental to calcium homeostasis when calcium and vitamin D are consumed at recommended intakes.

Ample evidence suggests that the current RDA for protein is inadequate to maintain optimal health, particularly during ED and for populations susceptible to bone loss (eg, the elderly) (34, 35). Because high-protein diets are increasingly used for weight loss in overweight and obese individuals, our findings refute a concern regarding the prospect of a negative effect of these diets on bone health (15–18).

Consistent with other studies (19, 22, 23), the dietary protein level was associated with increased urinary titratable acidity and decreased pH, likely reflecting the increase in estimated dietary PRAL. Urinary calcium excretion was not affected by dietary protein level in the current study but followed the expected trend with our previous report (23). The calcuretic effect of dietary

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Urinary measurements in healthy volunteers consuming controlled diets containing different levels of protein during the 10-d WM and 21-d ED diets.</th>
<th>2 × RDA (n = 10 M, 5 F)</th>
<th>3 × RDA (n = 10 M, 5 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WM</td>
<td>ED</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>149.6 ± 21.4</td>
<td>209.9 ± 21.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>97.9 ± 9.7</td>
<td>106.3 ± 7.7</td>
<td>0.268</td>
</tr>
<tr>
<td>Nitrogen (g/d)</td>
<td>9.5 ± 1.1</td>
<td>11.3 ± 1.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Ammonium (nmol/d)</td>
<td>21.4 ± 3.1</td>
<td>31.4 ± 3.2</td>
<td>0.365</td>
</tr>
<tr>
<td>Chlorine (mg/d)</td>
<td>157.8 ± 12.4</td>
<td>130.6 ± 10.0</td>
<td>0.345</td>
</tr>
<tr>
<td>NTX (nmol BCE)</td>
<td>57.0 ± 9.4</td>
<td>66.6 ± 11.9</td>
<td>0.720</td>
</tr>
<tr>
<td>Sulfate (mg/d)</td>
<td>14.9 ± 3.2</td>
<td>13.2 ± 2.9</td>
<td>0.164</td>
</tr>
<tr>
<td>pH</td>
<td>6.63 ± 0.06</td>
<td>6.30 ± 0.09</td>
<td>0.002</td>
</tr>
<tr>
<td>Titratable acid (mEq/d)</td>
<td>10.3 ± 3.0</td>
<td>21.8 ± 3.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. BCE, bone collagen equivalents; DPD, deoxypyridinoline; ED, energy deficit; NTX, amino-terminal cross-linking telopeptide of bone collagen; RDA, Recommended Daily Allowance. Mixed-model ANOVA was used to determine the main effects of dietary protein and dietary phase. Bonferroni correction was used to determine the main effects of dietary protein and dietary phase.
Protein has been attributed to its metabolic acidity or its contribution to dietary PRAL—a measure of the acid-base load of foods that can be used to estimate renal net acid excretion (27, 32, 36) rather than protein, per se. In support of this hypothesis, several studies have directly shown that supplementing the base-forming mineral potassium bicarbonate decreases urinary calcium excretion (37–40). Therefore, the nonsignificant difference in urinary calcium excretion was likely due to a smaller difference in dietary PRAL between the 3 protein levels (<40 mEq/d) compared with our earlier study (~80 mEq/d). Similarly, in a study with dietary protein contents ranging from 0.8 to 1.7 g/kg and a comparable PRAL difference (~32 mEq/d), the high-protein (mainly meat) diet did not significantly increase urinary calcium excretion, as measured with a radiotrace method despite elevated urinary acidity (19). The finding of no increase in urinary calcium excretion despite a reduced urinary pH may suggest that the modest acid load induced by consuming high-protein diets with mixed, high-quality sources was readily buffered by other systems (e.g., kidneys), thereby diminishing the buffering requirement on bone.

Although not statistically different, fractional calcium absorption changed in the anticipated direction of urinary calcium excretion. We recognized that our sample size may have contributed to the lack of statistical power; however, the apparent difference in fractional calcium absorption when compared with our earlier study was likely due to a smaller difference in urinary calcium excretion was likely due to a smaller difference in dietary PRAL between the 3 protein levels (<40 mEq/d) compared with our earlier study (~80 mEq/d). Similarly, in a study with dietary protein contents ranging from 0.8 to 1.7 g/kg and a comparable PRAL difference (~32 mEq/d), the high-protein (mainly meat) diet did not significantly increase urinary calcium excretion, as measured with a radiotrace method despite elevated urinary acidity (19). The finding of no increase in urinary calcium excretion despite a reduced urinary pH may suggest that the modest acid load induced by consuming high-protein diets with mixed, high-quality sources was readily buffered by other systems (e.g., kidneys), thereby diminishing the buffering requirement on bone.

Data showing no effect of protein intake levels on calcium retention (the difference in calcium absorbed from the diets and excreted in urine) do not represent whole-body calcium balance because neither fecal calcium excretion nor sweat calcium loss was measured. Nevertheless, the evidence further supports our conclusion that high-protein diets are not detrimental to calcium metabolism.

In this study, increasing the protein level did not result in elevated serum IGF-I and decreased serum PTH concentrations in healthy young adults—findings different from several previous studies with adults (22, 23, 42–45). IGF-I is an anabolic osteotropic agent that imparts an important role in bone metabolism by increasing osteoprogenitor proliferation and differentiation (46). Conversely, serum PTH regulates calcium homeostasis by increasing bone resorption and stimulating intestinal calcium absorption and renal calcium reabsorption to maintain normal blood calcium concentrations (47). The discrepancy between study populations is likely a result of an age-associated increase in PTH and decrease in IGF-I (48).

Decreased IGF-I coupled with increased TRAP, a marker of bone resorption, during ED compared with WM may indicate decreased bone formation and increased bone resorption in response to short-term ED. These results are consistent with reports showing that reduced IGF-I after caloric restriction (52–54) and increased markers of bone resorption subsequent to weight reduction (55–57). For example, a 5% reduction in body weight by dietary restriction alone or through the combination of dietary restriction and exercise resulted in decreased serum C-terminal telopeptide, also a marker of bone resorption, in overweight premenopausal women (56).

Given the relatively short duration of the study, our finding of no difference in BMD between the WM and ED phases—despite an average weight loss of 3.2 ± 0.2 kg during the latter phase (25)—should not be interpreted as indicating that the type and amount of physical activity prescribed in the study completely compensated for bone loss induced by weight loss in the long term. Whether exercise can offset the detrimental effects of energy restriction on bone remains unclear (58, 59) and may depend on many factors, such as the severity of the ED and the amount and type of physical activity performed. Although physical activity was tightly controlled, this study was not designed to determine the influence of physical activity on bone metabolism.

Because the study was conducted in Grand Forks, ND (47°55′ N), and spanned >1 y, each subject received a multivitamin-mineral supplement containing 400 IU vitamin D during the study to ensure comparable vitamin D status across study subjects. On the basis of serum 25(OH)D concentrations, the vitamin D status of the subjects in our study was normal (60). Our observation that ED was associated with higher serum 25(OH)D concentrations is consistent with other reports (61, 62), likely because of its release from adipose tissue (63). However, the
implication of enhanced vitamin D status with ED on bone metabolism is unclear.

In summary, consumption of high-protein diets up to 3 times the RDA in healthy young adults did not negatively affect calcium homeostasis or indexes of bone turnover. Although reduced IGF-I and elevated TRAP concentrations may indicate increased bone turnover, the near significant increase in calcium absorption coupled with no detectable changes in BMD, BMC, and urinary calcium excretion in response to short-term ED suggest that a long-term study is needed to determine whether weight loss induced by the combination of diet and exercise is detrimental to bone health. Regardless, these results combined with our previous findings that high-protein diets spare muscle protein (25) support the use of high-protein diets for healthy physically active adults who may undergo planned or unavoidable periods of ED.

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The authors’ responsibilities were as follows—JJC, SMP, LMM, LDW, JPM, AJY, and GFC: contributed to the study design, study implementation, data analysis and interpretation, manuscript preparation, or critical review of the manuscript; LMM: contributed to coordination of the study, data collection, and data management; and ERS: contributed to calcium isotope administration and medically supervised the study subjects. None of the authors declared any personal or financial conflicts of interest. The USDA Agricultural Research Service and the US Army Medical Research and Material Command had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

REFERENCES