A Diet High in Protein, Dairy, and Calcium Attenuates Bone Loss over Twelve Months of Weight Loss and Maintenance Relative to a Conventional High-Carbohydrate Diet in Adults

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Abstract

Weight loss causes bone mineral loss. Higher protein diets continue to be criticized for further potential harmful bone effects, including elevated urinary calcium, but may promote bone health if protein sources include dairy. Overweight middle-aged subjects (n = 130, 59 males) were randomized to a diet providing 1.4 g·kg⁻¹·d⁻¹ protein and 3 daily servings of dairy (PRO) or 0.8 g·kg⁻¹·d⁻¹ protein and 2 daily servings of dairy (CARB) for 4 mo of weight loss plus 8 mo of weight maintenance. Diets prescribed 6276 kJ/d for females and 7113 kJ/d for males. Bone mineral content and density (BMD) for whole body (WB), lumbar spine (LS) and total hip (TH) were measured using dual X-ray absorptiometry, and dietary intake using 3-d weighed food records. Urinary calcium was measured using 24-h collection at 0 and 8 mo for a subsample (n = 42). Participants lost body weight (mean, 95% CI) of 8.2% (7.5–8.9%) at 4 mo, 10.6% (9.5–11.8%) at 8 mo, and 10.5% (8.9–12.0%) at 12 mo without differences between groups at any time (P = 0.64). At 12 mo, PRO BMD was higher by 1.6% (0.3–3.0%) at WB, 2.1% (0.6–3.7%) at LS, and 1.4% (0.2–2.5%) at TH compared with CARB. PRO calcium intake was higher (PRO: 1140 ± 658 mg/d, CARB: 766 ± 46; P < 0.01), as was urinary calcium (PRO: 163 ± 15 mg/d, CARB: 100 ± 9.2; P < 0.01). A reduced-energy diet supplying 1.4 g·kg⁻¹·d⁻¹ protein and 3 dairy servings increased urinary calcium excretion but provided improved calcium intake and attenuated bone loss over 4 mo of weight loss and 8 additional mo of weight maintenance. J. Nutr. 138: 1096–1100, 2008.

Introduction

Weight loss has well-established, favorable effects on metabolic disease risk such as type 2 diabetes mellitus and cardiovascular disease in overweight populations (1); however, weight loss also promotes loss of bone mass and increases fracture risk (2–4). As an aging population confronts concurrent threats of obesity and osteoporosis, diets that promote weight loss while maintaining bone mineral mass and density are of special interest.

Higher protein weight loss diets have received attention due to purported improvements in adherence and body composition, including enhanced loss of fat mass and preservation of lean mass (5–7). Recent prospective and clinical trials suggest that higher protein diets, if accompanied by adequate calcium, enhance bone health (8–11). This remains controversial in light of long-standing theory and evidence that increasing protein intakes promote calciuria (12). Increased urinary calcium with greater protein intake is traditionally considered to reflect bone demineralization; however, Kerstetter et al. (13) have shown that additional dietary protein promotes intestinal calcium absorption and reduces the fraction of urinary calcium of bone origin. Dawson-Hughes (14) has proposed that the net effect of dietary protein on bone mineral status depends on dietary availability of calcium.

In light of these observations, we propose that a diet utilizing dairy foods as a source of both protein and calcium will preserve bone mineral density (BMD) and content (BMC) relative to a...
conventional higher carbohydrate weight loss diet. We have adopted a free-living, freely selected diet approach in which patients are educated about selection of food groups rather than directly controlling intakes of individual nutrients. This design tests effectiveness, rather than efficacy. That is, it will not resolve the independent or interactive effects of dietary protein and calcium on bone health during weight loss; however, the design is more generalizable to clinical practice than highly controlled feeding studies and more directly answers the question most relevant to the clinical practitioner: “What is the effect on bone health of prescribing a higher protein weight loss diet emphasizing dairy?”

The primary aim of this study was to compare BMC and BMD during 4 mo of active weight loss and 8 additional mo of weight maintenance in free-living participants on a diet providing 1.4 g·kg\(^{-1}·d\(^{-1}\) protein and 3 dairy servings (PRO) compared with an isocaloric, conventional weight loss diet providing 0.8 g·kg\(^{-1}·d\(^{-1}\) protein (CARB). Based on previous work, we anticipated increased protein and calcium intakes, elevated urinary calcium excretion, and attenuated bone loss in PRO compared with CARB participants.

**Subjects and Methods**

**Subjects and design.** A total of 130 subjects (59 males) aged 30 and 65 y (mean ± SD, 45.6 ± 8.9 y) were recruited to a 12-mo randomized, parallel-arm, multi-center (Illinois and Pennsylvania sites) weight loss trial. Exclusion criteria were as follows: BMI < 26 kg/m\(^2\); body weight > 140 kg due to constraints of the dual X-ray absorptiometry (DXA) scanning bed; smoking and conditions or medications affecting study outcomes, including cancer, heart disease, diabetes mellitus, renal disease or insufficiency; major weight change in the prior 6 mo, or use of bone active medications or supplements, oral steroids, or antidepressants. Participants were blocked into groups of similar age and BMI, then blocks were randomly divided into PRO or CARB diets. Institutional Review Boards of the University of Illinois at Urbana-Champaign and The Pennsylvania State University approved all methods; subjects provided written informed consent.

**Treatments.** The PRO diet prescribed 1.4 g·kg\(^{-1}·d\(^{-1}\) protein, 3 daily servings of dairy, and ~30, 40 and 30% of energy, respectively, from protein, carbohydrate, and fat (carbohydrate:protein ratio < 1.5). The CARB diet prescribed 0.8 g·kg\(^{-1}·d\(^{-1}\) protein, 2 daily servings of dairy, and roughly 15, 55, and 30% of daily energy, respectively, from protein, carbohydrate, and fat (carbohydrate:protein ratio > 3.5). Both diets prescribed 6276 kJ/d for females and 7113 kJ/d for males, and equal total fat (~57 g/d) and fiber (~17 g/d). Subjects were asked not to take any dietary supplements during the study.

Research dietitians instructed participants on portion sizes and emphasized the Food Guide Pyramid (15) for the CARB group and replacement of starchy staples (breads, rice, pasta, cereals) with meats, eggs, and dairy for the PRO group. Participants were provided 2-wk cycling menu plans. Each diet prescribed micronutrient intakes meeting recommended dietary allowances (16), fat intake meeting guidelines by the AHA (17), and 5 servings of vegetables and 2-3 servings of fruit daily. Participants reported 1 h weekly for support, questions and answers, review of diet records and compliance, submission of 3-d weighed food records, and body weight measurement. Mean intakes at 0, 4, 8, and 12 mo were calculated with Nutritionist Pro software (First DataBank) and means were used for analysis of dietary compliance and nutrient intakes. We encouraged participants to spend at least 30 min walking 5 d/wk according to NIH Guidelines for Weight Management (1). Activity was monitored using daily logs and 3 d/week using armband accelerometers (BodyMedia).

**Bone and body composition.** Whole body (WB), lumbar spine (LS), and total hip (TH) DXA scans were performed at 0, 4, 8, and 12 mo (Illinois: Hologic QDR 4500A, software version 11.1.3; Penn State: Hologic QDR 4500W, software version 12.5). Scans for a given individual were analyzed by the same technician at each site using standard manufacturer guidelines. The same array scan mode was used for all central measure scans. DXA machines were calibrated daily using manufacturer phantoms. Analyzed LS data included L1–L4. Volunteers wore light, metal-free, cotton clothing. CVs for DXA outcomes of interest are 1.0–2.0%.

**Urinary calcium measures.** Twenty-four-hour urine samples were collected at baseline and at 8 mo from Illinois participants. A total of 42 Illinois participants remained in the study at 8 mo, providing complete urinary calcium data. Ten-milliliter aliquots of mixed collections were diluted and tested by atomic flame absorption spectroscopy, using a Perkin-Elmer 360 atomic absorption spectrophotometer to determine calcium concentration.

**Statistical analysis.** We used SPSS version 14 for all statistical analyses. Normality assumptions were tested by the Shapiro-Wilk statistic. Reported values are means ± SEM for normally distributed variables and median [interquartile range (IQR)] for non-normally distributed variables. Baseline characteristics, weight change, and intakes were compared using ANOVA. Linear mixed models with random slopes and time of measurement as a repeated effect were applied to BMC and BMD at the 3 measurement sites (WB, LS, and TH) in intent-to-treat fashion, adjusted for baseline values and gender, site of study participation, and 2- and 3-way interactions of gender and study site with diet and time. We also tested terciles of age among female participants to indirectly control for menopausal status. Dietary treatment effects on urinary calcium measurements at 8 mo were analyzed using ANCOVA, controlling for baseline urinary calcium and gender. ANCOVA was also employed to assess whether elevated urinary calcium excretion was related to BMC within dietary treatment groups. Additional details of the statistical analysis are in the supplemental appendix. Values in the text are means (95% CI) or means ± SEM.

**Results**

At baseline, groups did not differ except that PRO females had lower WB BMC than CARB females (Table 1) and CARB males reported smaller energy and macronutrient intakes than PRO males (Table 2; Supplemental Table 1). One extreme, lone outlier (sample Z-score ≤ −3.5 or ≥3.5 without skew) was identified in both BMC and BMD at the WB and TH, and 2 extreme outliers at the LS. Examination revealed no clear reason for their departure from predictions; they were excluded from analysis. In the PRO group, 12, 6, and 5 participants withdrew from the intervention at 4, 8, and 12 mo, respectively; in the CARB group, 14, 14, and 7 participants withdrew, respectively.

Participants lost 8.2% (7.5–8.9%) of their initial body weight at 4 mo and 10.5% (8.9–12.0%) at 12 mo with no differences between the diet groups (Supplemental Table 2). By 4 mo, energy intake was centered approximately at prescribed levels and protein and calcium intakes diverged according to diet as prescribed (diet × time interaction, P < 0.05; Table 2). Energy intake did not differ by diet through the intervention (Table 2). At 4 mo, protein intake was 1.37 ± 0.04 g·kg\(^{-1}·d\(^{-1}\) or 29 ± 0.6% of energy in the PRO group and 0.82 ± 0.03 g·kg\(^{-1}·d\(^{-1}\) or 18 ± 0.3% of energy in the CARB group, indicating compliance to the prescribed dietary treatments. Protein intake at 12 mo was comparable (Table 2). By 12 mo, fat intake was slightly greater than at 4 mo in both diet groups (P < 0.05 (Supplemental Table 2).

As expected, an increase in protein intake by PRO participants replaced predominantly carbohydrate, although PRO participants also consumed slightly more fat (Supplemental
Calcium intake increased in the PRO group and declined in the CARB group with energy restriction (Table 2). PRO participants consumed 387 ± 72 mg more calcium daily than CARB participants at 4 mo and 261 ± 81.6 mg at 12 mo (P, 0.01). While PRO calcium intakes met the recommended dietary allowance (16), calcium intake provided by the CARB diet was inadequate for female participants (Table 2). Because protein and calcium intakes increased or decreased together according to diet assignment, the ratio of protein:calcium intakes did not differ by diet or time (Supplemental Table 2).

Mean servings of dairy were as prescribed: 2 servings per day in CARB and 3 in PRO participants. Subjects reported no intake of supplements, as prescribed. Food records indicated that PRO participants consumed 169 ± 16 IU vitamin D, which was 34 ± 23 IU more than CARB participants (P = 0.15); however, our nutrient database was not complete for all foods with respect to vitamin D, and actual contents may vary considerably from labeled values (18,19), making the accuracy of these estimates questionable. Physical activity did not differ between diet groups. BMD at the WB, LS, and TH was greater over the course of the study in PRO participants (Fig. 1). BMC was also greater in PRO participants (P < 0.05; Supplemental Table 3).

Treatment differences were similar across gender and its 2- and 3-way interactions with diet and time for all bone outcomes (P > 0.05). Gender differences in BMD could be predicted by preintervention status; therefore, gender effects were not significant after controlling for baseline values (P > 0.10), indicating males and females were similarly affected by the diets. Similarly, tests of age group (<40, 40–50, >50 y) and its interactions with diet and time in females were not significant, indirectly suggesting menopausal status did not moderate response.

Urinary calcium declined by mo 8 in the CARB group but was maintained in the PRO diet group (Fig. 2). Adjusting for baseline BMC, diet group, and gender, urinary calcium levels predicted decreased WB BMC (b = −0.38; P = 0.032), indicating that a
portion of variation in urinary calcium that was not explained by protein intake was negatively associated with WB BMC. This effect was not observed for LS BMC ($P = 0.65$) or TH BMC ($P = 0.59$).

**Discussion**

After a combined 12 mo of weight loss (4 mo) and maintenance (8 mo), a weight loss diet prescribing 1.4 g kg$^{-1}$d$^{-1}$ protein and 3 daily servings of dairy provided more calcium and preserved bone mineral relative to an isocaloric control diet prescribing 0.8 g kg$^{-1}$ d$^{-1}$ protein and 2 daily servings of dairy. Targeting lean protein sources, including low-fat dairy, improved calcium intake in free-living individuals under reduced energy intake conditions, whereas a conventional, high-carbohydrate diet provided inadequate calcium in females, consistent with other reports (20,21). These data indicate that a higher protein diet specifically emphasizing dairy preserves bone loss compared with an isonenergetic higher carbohydrate diet proportioned according to the Food Guide Pyramid. Our data do not permit the resolution of independent or interactive effects of protein and other dairy components such as calcium and vitamin D but support the effectiveness of a high-protein, high-dairy diet for protecting bone health during weight loss in free-living patients.

Shaples and Riedt (4) reviewed reports of bone mineral loss with weight loss and summarize that 10% weight loss would be expected to produce 1–2% bone loss at various sites. Loss of BMD with weight loss is thought to occur due to changes in the weight-bearing load, estrogen status, circulating leptin, and reduced calcium intake with energy restriction. Skov et al. (8) reported that a higher protein diet conferred greater weight loss but similar bone loss or an improved bone loss:weight loss ratio over 6 mo in adults. As in the present study, Skov et al. (8) reported adequate calcium intakes on the higher protein diet but low calcium intakes among higher carbohydrate dieters.

The influence of protein intake on calcium balance and bone health remains controversial. Higher protein intakes consistently increase urinary calcium excretion (22). Early research found no connection between calcium absorption and protein intake (23–26), suggesting extra urinary calcium must reflect loss from bone (12). Specifically, it is thought that bone is demineralized to buffer acid reabsorbed during renal handling of sulfate metabolites of dietary methionine and cysteine (27). More recently, Kerstetter et al. (28–30) have reported increased calcium absorption with higher protein intakes using dual stable calcium isotopes and calcium-controlled interventions; however, a similar study did not find elevated absorption based on protein intake (31). Roughhead et al. (32) reported no differences but a trend toward higher calcium retention in a group consuming increased protein from whole-food (meat) sources. Critically, bone metabolism markers did not differ. Another study demonstrated an increase in the fraction of urinary calcium of dietary origin during a diet providing 2.1 g kg$^{-1}$ d$^{-1}$ protein compared with a control diet providing 1.0 g kg$^{-1}$ d$^{-1}$ (13), suggesting additional excreted calcium did not originate in bone but rather from improved intestinal absorption.

Dawson-Hughes et al. (33) reported a calcium-protein interaction in participants randomized to calcium supplementation or placebo and divided into tertiles of freely selected protein intake. Elderly participants consuming more protein and calcium gained bone mineral relative to those at lower intakes of either nutrient. Bowen et al. (11) measured bone resorption and formation markers across 12 wk of weight loss on a mixed protein or dairy protein weight-loss diet, concluding that the calcium-rich, dairy-based diet reduced bone turnover compared with a diet rich in protein but poorer in calcium. In the present study, ample calcium was supplied as a natural consequence of emphasizing lean protein sources, including dairy.

Median daily urinary calcium was 167 mg for PRO and 98 mg for CARB dieters. PRO males consumed ∼400 mg and females 300 mg more calcium daily than CARB participants. Accordingly, assuming a conservative 20–25% calcium absorption, additional available calcium would approximately compensate or exceed urinary losses. We have no data to account for endogenous fecal calcium losses, which may also be influenced by protein intake (34), nor can we report actual calcium absorption. However, if absorption increases with protein as reported by Kerstetter et al. (13,28–30), gains in available calcium could substantially outweigh urinary losses, supporting bone mineralization. Our data support this speculation.

Our study is not without limitations. Compliance assessment relies on 3-d weighed food records. Available nutrient databases are incomplete with respect to vitamin D, making inference concerning this nutrient difficult. DXA measurement is influenced by changes in tissue thickness, an effect that is not well quantified (35,36). Accordingly, changes over time within diet groups should be interpreted with care; however, the relative impact of the PRO vs. CARB diet is interpretable, as groups did not differ in weight, fat, or body thickness change. All measures demonstrated a marked benefit of the PRO compared with the CARB diet on the order of 1 to 3%. The independent effects of increased protein and calcium on bone health are not known given the present design. Although limiting internal validity (i.e. isolation of protein and calcium effects), the design maximizes external validity, demonstrating that a high-dairy, high-protein diet will protect, not harm, bone relative to a conventional weight loss diet in free-living patients.

In conclusion, a higher protein weight loss diet emphasizing dairy as a lean protein source naturally improved calcium intake and preserved bone mineral during weight loss relative to a conventional higher carbohydrate diet in this free-living population. Though not observed in the present sample, it is likely
that such a diet would improve vitamin D intake for some patients due to fortification of this nutrient in dairy, conferring further bone benefits. These data are consistent with current literature indicating dietary protein may have direct benefits to bone health, provided calcium intake is adequate (8–10,33). Obesity and osteoporosis are major public health concerns and are at odds with one another; to treat the first is often inviting the other. A dietary regimen that protects bone health while promoting fat loss is of high clinical value.

**Literature Cited**


