The Journal of Nutrition
Biochemical, Molecular, and Genetic Mechanisms

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Partial Replacement of Animal Proteins with Plant Proteins for 12 Weeks Accelerates Bone Turnover Among Healthy Adults: A Randomized Clinical Trial

Suvi T Itkonen,[1] Essi Päivärinta,[1] Tiina Pellinen,[1] Hanna Viitakangas,[1] Juha Risteli,[2] Maijaliisa Erkkola,[1] Christel Lamberg-Allardt,[1] and Anne-Maria Pajari[1]

[1]Department of Food and Nutrition, University of Helsinki, Helsinki, Finland; and [2]Cancer and Translational Medicine Research Unit, Department of Clinical Chemistry, University of Oulu, Oulu, Finland

ABSTRACT

Background: Plant-based diets may reduce the risk of chronic diseases, but can also lead to low calcium and vitamin D intakes, posing a risk for bone health.

Objectives: We investigated whether partial replacement of animal proteins with plant-based proteins using a whole-diet approach affects bone and mineral metabolism in healthy adults in 3 groups fed diets differing in protein composition.

Methods: This 12-week clinical trial was comprised of 107 women and 29 men (20–69 years old; BMI mean ± SD, 24.8 ± 3.9) randomly assigned to consume 1 of 3 diets designed to provide 17 energy percent (E%) protein: "animal" (70% animal protein, 30% plant protein of total protein intake), "50/50" (50% animal, 50% plant), and "plant" (30% animal, 70% plant) diets. We examined differences in bone formation [serum intact procollagen type I amino-terminal propeptide (S-iPINP)], bone resorption [serum collagen type 1 cross-linked C-terminal telopeptide (S-CTX)], mineral metabolism markers (primary outcomes), and nutrient intakes (secondary outcomes) by ANOVA/ANCOVA.

Results: S-CTX was significantly higher in the plant group (mean ± SEM, 0.44 ± 0.02 ng/mL) than in the other groups (P values < 0.001 for both), and differed also between the animal (mean ± SEM, 0.29 ± 0.02 ng/mL) and 50/50 groups (mean ± SEM, 0.34 ± 0.02 ng/mL; $P = 0.018$). S-iPINP was significantly higher in the plant group (mean ± SEM, 63.9 ± 1.91 ng/mL) than in the animal group (mean ± SEM, 55.0 ± 1.82 ng/mL; $P = 0.006$). In a subgroup without a history of vitamin D supplement use, plasma parathyroid hormone was significantly higher in the plant than in the animal group ($P = 0.018$). Vitamin D and calcium intakes were below recommended levels in the plant group (mean ± SEM, 6.2 ± 3.7 μg/d and 733 ± 164 mg/d, respectively).

Conclusions: Partial replacement of animal proteins with plant-based proteins for 12 weeks increased the markers of bone resorption and formation among healthy adults, indicating a possible risk for bone health. This is probably caused by lower vitamin D and calcium intakes from diets containing more plant-based proteins, but it is unclear whether differences in protein intake or quality play a major role. This trial was registered at clinicaltrials.gov as NCT03206827. *J Nutr* 2021;151:11–19.

Keywords: bone turnover, mineral metabolism, plant protein, animal protein, calcium, vitamin D, clinical trial

Introduction

The EAT-Lancet report suggests increased consumption of plant-based foods and decreased consumption of animal-based foods to attain a healthy diet and sustainable food production (1). Plant-based diets may reduce the risk of some chronic diseases, such as cardiovascular disease, type 2 diabetes, and colorectal cancer, through their nutritional benefits, which include higher fiber and folate contents and better fat quality (2–4). However, plant-based diets can lead to low intakes of such nutrients as calcium and vitamin D, which are important for bone health (5). Low vitamin D status leads to decreased calcium absorption and higher parathyroid hormone (PTH) concentrations, potentially causing bone loss (6). A recent systematic review and meta-analysis of 20 studies including 37,134 participants showed that vegetarians and vegans had lower lumbar spine and femoral neck bone mineral density and higher fracture risks than omnivores (7).

Adequate intake of protein is necessary for maintaining bone health throughout life (8). Dietary protein and amino acids can modify calcium and bone metabolism by modulating

Manuscript received March 6, 2020. Initial review completed April 6, 2020. Revision accepted August 6, 2020.
First published online September 16, 2020; doi: https://doi.org/10.1093/jn/nxaa264.

calcium absorption and excretion and changing hormonal and growth factor regulation of bone and bone cell functions (9). Dietary protein enhances insulin-like growth factor I (IGF-I) production, which stimulates 1,25-dihydroxyvitamin D synthesis, increasing calcium and phosphate absorption, as well as tubular phosphate reabsorption (8). Dietary protein can also suppress PTH concentration, which is an important regulator of bone turnover (8). Amino acids can activate calcium-sensing receptors and increase calcium translocation in the intestinal epithelium. Enhanced calcium absorption by higher protein intake may explain increased calciuria without negative calcium balance (8). Sulfurous, animal-derived acidic amino acids, such as cysteine and methionine, have earlier been suggested to increase bone loss through accelerated calcium excretion caused by the acidic environment (10). However, later studies have revealed that high protein intake and dietary acid load have no harmful effects on calcium metabolism (8, 10). In vitro studies have demonstrated that different amino acids have specific mechanisms on bone metabolism (11–13). Such amino acids as arginine, lysine, alanine, leucine, proline, and glutamine can stimulate insulin secretion, promoting osteoblast growth and differentiation (13, 14). Arginine stimulates growth hormone production and, therefore, IGF-I production (11). Arginine and lysine also have positive effects on collagen production and synthesis (12). In humans, ingestion of the nonsulfurous amino acids arginine and lysine in a low-protein diet has also shown a trend to increase calcium absorption, whereas no effects of phenylalanine, histidine, or tryptophan were observed (15). Thus, different protein sources (plant and animal) with varying amino acid profiles could have diverse effects on bone. Vegetarian and vegan diets comprising a variety of foods are suggested to provide adequate intake of all amino acids, but lysine may be the limiting amino acid in monotonous vegan diets containing cereals as the main or sole source of protein (16). However, the meta-analyses and systematic reviews of intervention, observational, and prospective studies of the effects of animal and plant protein sources on bone health (bone mineral density, fracture risk) have not found any protein sources to be more advantageous than others (10, 17, 18). Previous intervention studies regarding the effects of plant-based proteins/diets versus animal-based proteins/diets on mineral metabolism have been mainly carried out among kidney patients, and the results are somewhat inconsistent, but plant-based proteins are generally preferred for this patient group (19–21).

To our knowledge, no controlled, long-term intervention studies on the effects of replacing animal protein with plant-based protein on bone and mineral metabolism have been carried out among healthy people. Overall, data on the health effects of designed flexitarian diets containing some animal-based protein sources are scarce. As adherence to a more plant-based diet may cause a risk for bone health, in this study we aimed to investigate the effects of partial replacement of animal-based protein sources with plant-based protein sources on bone turnover and mineral metabolism in healthy adults in a 12-wk randomized clinical trial.

Methods

Study design

The ScenoProt intervention study was a randomized clinical trial conducted at the Department of Food and Nutrition, University of Helsinki, Finland, between December 2016 and June 2017 (22). The study was carried out in a parallel design with 3 equal-sized intervention groups following diets containing different proportions of animal- and plant-based protein sources. The trial was a part of the large, multidisciplinary research project "ScenoProt—Novel protein sources for food security." The study was registered at clinicaltrials.gov as NCT03206827 and was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (1651/2016). Written informed consent was obtained from all subjects.

Study participants

The participants were recruited using newspaper advertisements, mailing lists, the intranet of the University of Helsinki, and a social media platform (Facebook). Eligibility criteria for participants were being healthy and omnivorous, being willing to follow a randomly allocated intervention diet for 12 wk, an age of 20–69 y, and having a BMI of 18.5–35.0 kg/m^2. Exclusion criteria were having inflammatory bowel diseases, irritable bowel syndrome, or coeliac disease; taking medication for diabetes or hypercholesterolemia; having a disorder of the endocrine system or lipid metabolism; having liver or renal diseases; having cancer within the past 5 y; regular or recent (within 3 mo) use of antibiotics; regular use of nutritional supplements; having food allergies; having an eating disorder; doing extreme sports; smoking; or pregnancy or lactation. All volunteers meeting the criteria were asked for written informed consent, and thereafter were invited to a screening test to analyze their plasma total cholesterol and glucose after an overnight fast (10–12 h). Participants with plasma total cholesterol ≤6.5 mmol/L and glucose ≤6.9 mmol/L were invited to attend the intervention period. Of the 179 Caucasian participants screened, 145 were stratified by sex and age, and randomly assigned to 1 of 3 intervention diets for 12 wk (**Figure 1**). Participants were advised to discontinue the use of nutritional supplements and natural products 2 wk prior to the beginning of the intervention period. They were requested to maintain physical activity at their usual level throughout the study.

Intervention diets

The basis of the intervention diets is described in **Supplemental Table 1**, and the diets are described in detail elsewhere (22). The intervention diets were designed following the principles of the whole-diet approach (23), taking into account the potential synergistic or additive effects that components of the diet may have. As plant protein sources inherently contain other nutrients than protein (e.g., fiber), the replacement of animal protein sources with plant protein ones leads to changes in the overall composition of the diet. The protein intake in all diets was designed to be 17 E%, while the proportions of animal- and plant-based proteins differed as follows; "animal" refers to an animal protein–based diet representing an average Finnish diet (24) and containing 70% of protein from animal sources (red and white meat, fish, eggs, dairy products) and 30% from plant sources (mainly bread, other cereals, potatoes); "50/50" refers to a diet containing equal proportions (50:50)

This study was funded by the Strategic Research Council at the Academy of Finland (grant numbers 293045 and 314243), the Jenny and Antti Wihuri Foundation, the Emil Aaltonen Foundation, and Medicinska Understödsföreningen Liv och Hälsa.

Author disclosures: STI, EP, TP, HV, JR, ME, CL-A, A-MP, no conflicts of interest.

Supplemental Tables 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

Address correspondence to STI (e-mail: suvi.itkonen@helsinki.fi).

Abbreviations used: CTX, collagen type 1 cross-linked C-terminal telopeptide; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; IGF-I, insulin-like growth factor I; iPINP, intact procollagen type I amino-terminal propeptide; P-25(OH)D, plasma 25-hydroxyvitamin D; P-iFGF23, plasma intact fibroblast growth factor 23; P-iPTH, intact parathyroid hormone; PTH, parathyroid hormone; S-Ca, serum calcium; S-Crea, serum creatinine; S-CTX, serum collagen type 1 cross-linked C-terminal telopeptide; S-iPINP, serum intact procollagen type I amino-terminal propeptide; S-Pi, serum phosphate; U-Ca, urinary calcium; U-Pi, urinary phosphate; 25(OH)D, 25-hydroxyvitamin D.

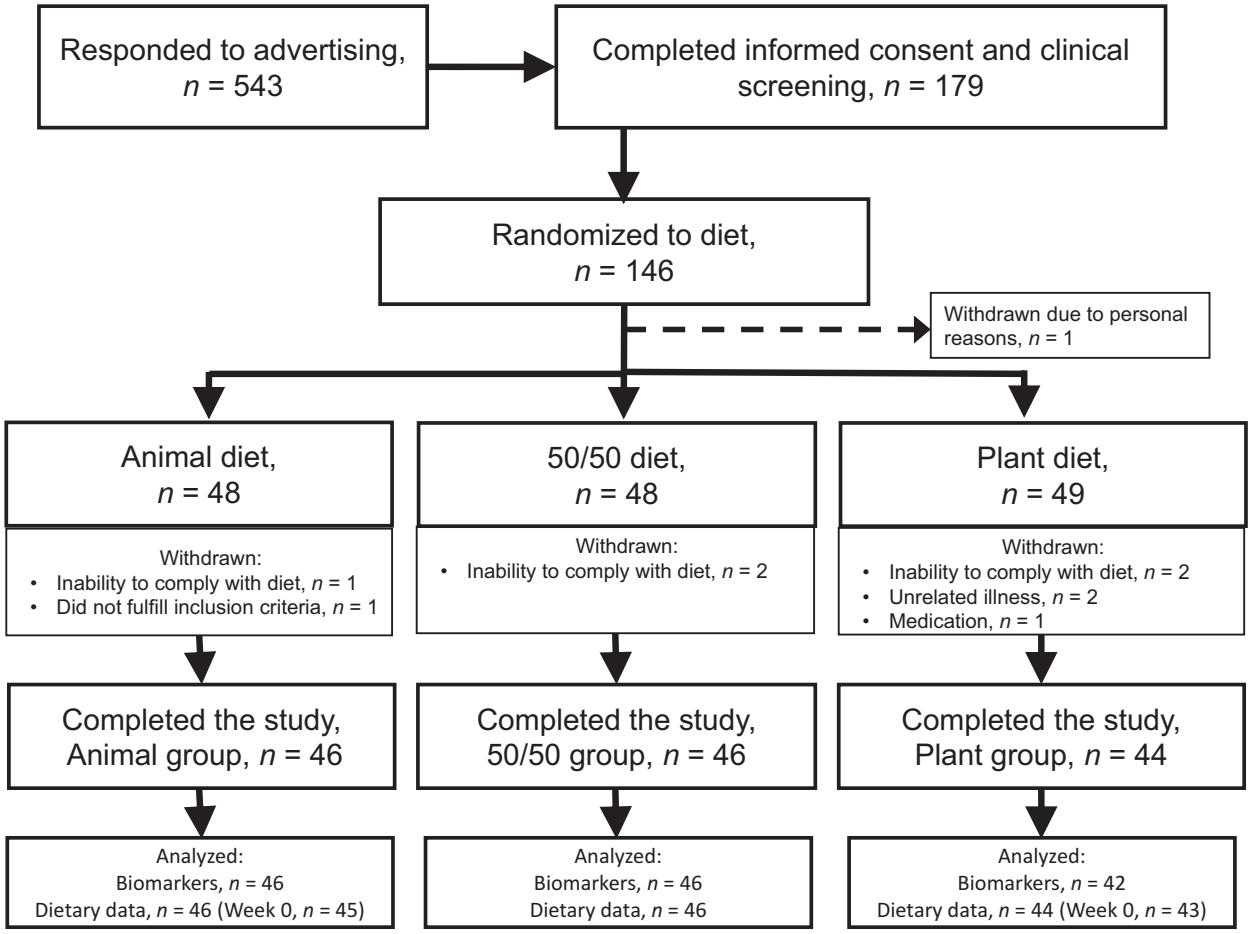

FIGURE 1 Flow chart of participants. The animal diet contained 70% animal-based and 30% plant-based protein sources of total protein intake; the 50/50 diet contained 50% animal-based and 50% plant-based protein sources of total protein intake; and the plant diet contained 70% plant-based and 30% animal-based protein sources of total protein intake.

of animal- and plant-based protein sources, but no more than 500 g of red and processed meat per week; and "plant" refers to a plant protein–based diet with 30% animal and 70% plant-based proteins. In the 50/50 and plant diets, animal-based protein sources were partly replaced with both new and traditional plant-based protein sources (legumes, nuts, seeds, and ready-made plant protein products, such as pulled oats and plant-based drinks). All diets contained the same amount of fish (250 g/wk) and eggs (4 eggs, on average 220 g/wk), but the amounts of dairy products differed, being highest in the animal diet (Supplemental Table 1).

Participants were provided with most of their protein sources by the study to be consumed at home; these included meat and meat products, poultry products, fish and vegetable patties, ready-made meals, frozen or dried pulses and other legume-based products, nuts, seeds, bread, and cereals, and excluded dairy products and eggs, which the participants were advised to use according to the instructions given. Based on the food records, the controlled food items in the intervention diets supplied, on average, 80% of the daily energy intake (22). The participants were advised to use their usual amounts of spread on bread and of cooking fats. They were allowed to consume habitual amounts of foods with low protein content, such as fruits, vegetables, juices, confectioneries, and alcoholic beverages.

The energy contents of the intervention diets were based on the average energy consumption of 8400 kJ/d in Finns (24), but the participants were advised to maintain a stable weight and to eat according to their appetite. Since the participants were given both foods to be eaten as such, as well as food ingredients, they were instructed to implement the diet on a food level, and food recipes were also delivered to support the implementation.

Background data

Background data on age, physical activity, history of supplement use, and medication use were collected from all participants by paper questionnaire. Data on education were collected through a web-based questionnaire. The habitual frequency (times/wk) and type of moderate-intensity physical activity (e.g., running, dancing, other sweat-inducing workout) was asked using a nonvalidated questionnaire. Previous regular use of vitamin and mineral supplements, including the brand name but not the dose, was recorded. The data were dichotomized to previous user or nonuser of vitamin D supplements or multivitamins containing vitamin D. Due to potentially different effects on mineral metabolism, the use of hormonal medication (not including information on the type of medication) was classified into 4 groups according to the age of the subject [we assumed that subjects <51 y were premenopausal and ≥51 y were menopausal or postmenopausal (25)]: *1*) <51 nonuser; *2*) <51 user; *3*) ≥51 nonuser; and *4*) ≥51 user. Data on education were dichotomized to basic education or secondary/university-level education. Weight and height were measured at baseline and endpoint, and BMI was calculated as weight (kg)/height (m^2). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula (26).

Biochemical analyses: primary outcome

Fasting blood samples and 24-h urine samples were used to determine the primary outcomes, biochemical markers of bone turnover, and mineral metabolism. Serum intact procollagen type I amino-terminal propeptide (S-iPINP) and collagen type 1 cross-linked C-terminal telopeptide (S-CTX) concentrations were analyzed using an IDS-iSYS Multidiscipline Automated Analyzer (Immunodiagnostic Systems Ltd)

at NordLab Oulu and at the Department of Clinical Chemistry, University of Oulu, Oulu, Finland. The ratio of PINP/CTX was calculated to assess the ratio of bone formation to bone resorption. Concentrations of intact parathyroid hormone (P-iPTH) and 25-hydroxyvitamin D [P-25(OH)D] in the plasma were analyzed by the chemiluminescent microparticle immunoassay method of Abbott Architect (Abbott Laboratories) at Helsinki University Central Hospital Laboratories, Helsinki, Finland. Serum phosphate (S-Pi), calcium (S-Ca), and creatinine (S-Crea) and urinary phosphate (U-Pi) and calcium (U-Ca) concentrations were analyzed using a photometric method by Indiko automatic analyzer (Thermo Clinical Labsystems Oy) and plasma intact fibroblast growth factor 23 (P-iFGF23) concentrations by Immutopics ELISA kit (Immutopics Inc.) at the Department of Food and Nutrition, University of Helsinki, Finland. Urinary 24-h excretion of phosphate and calcium was calculated based on collection times and urinary volumes.

Accuracy was assessed using commercial, quality-control sera (S-Ca, S-Pi, S-Crea) and urine (U-Ca, U-Pi; Thermo Fisher Scientific Inc.). For these analyses, inter- and intra-CV percentages were <3.8%. For P-iPTH, P-25(OH)D, P-iFGF23, S-iPINP, and S-CTX assays, controls supplied by the manufacturers were used. The intra-CV% for P-iPTH and P-25(OH)D were ≤4.0%. The inter-CV% for P-25(OH)D was ≤7%. For P-iFGF23, the inter-CV% was <5.8% and the intra-CV% <3.9%, and for S-CTX and S-iPINP the inter-CV% were <5.3% and the intra-CV% <2.9%, respectively. The mean biases of low, normal, and high controls for S-iPINP were +6.3%, +1.5%, and +2.4%, respectively, and for S-CTX were −3.0%, −0.5%, and −1.2%, respectively. In addition, for P-25(OH)D and P-iPTH assays, accuracy was assessed using external quality control programs (Labquality's External Quality Assessment Schemes) twice a year, and P-iPTH accuracy was also assessed with UK NEQAS monthly. In all instances, adherence to the laboratory's quality assessment scheme was ensured.

Nutrient intake analyses: secondary outcome

Intakes of bone-related nutrients were the secondary outcome of this study. Habitual dietary intake data of the participants were collected using 4-d food records completed within 1 wk both prior to and during the last week of the intervention, including on 3 weekdays and 1 weekend day. The food records of 2 participants at baseline were not available [for details regarding other exceptions in the length of recording, see Päivärinta et al. (22)]. The participants were given both written and oral instructions for filling in food records and estimating portion sizes with household measures and food package labels. Food records were reviewed by a nutritionist, and any missing information was requested from participants. Nutrient intakes were calculated using AivoDiet software (version 2.2.0.1, Aivo Oy), based on the continuously updated Finnish food composition database Fineli, maintained by the Finnish Institute for Health and Welfare. Nutrient intakes were analyzed as a daily mean of each recording period. In this paper, the intakes of energy-yielding nutrients and of phosphorus, calcium, and vitamin D are reported. Molar calcium-to-phosphorus ratios are also described, as are the dietary sources of phosphorus, calcium, and vitamin D.

Sample size estimation and randomization

This study was based on the secondary outcomes of the ScenoProt intervention study, the main aim of which was to investigate the effects of replacing animal proteins with plant-based proteins on biomarkers for colorectal cancer. Sample size was determined based on the data of concentrations of fecal heme-derived N-nitroso compounds, analyzed in a previous 4-wk intervention study (clinicaltrials.gov NCT02469285). The power calculation estimated that a sample size of 50 per intervention group would be adequate to show a statistical difference between the dietary groups at the endpoint of the study with a 95% CI and statistical power of 0.80. No power calculations regarding bone turnover or mineral metabolism markers were carried out.

The participants started their 12-wk intervention period between January and March 2017. An equal number of participants in each diet group started the intervention period each week. Screening was continued until the beginning of March 2017, and eligible participants were randomized into the diet groups. The principal investigator generated the allocation sequence and randomly assigned the participants into intervention groups within a similar deviation of age and gender. Blinding was not possible due to the nature of the intervention; color codes were used to mark the diets.

Statistical analyses

Statistical analyses were performed using SPSS Statistics version 25 (IBM). Data are presented as means and either SDs or SEs. The normality and homogeneity of the data were verified and log-transformed to improve normality if needed. U-Pi, S-CTX, S-iPINP, PINP/CTX ratio, and P-iFGF23 data, as well as all nutrient data, were log-transformed. All tests were considered significant at $P < 0.05$. One-way ANOVA was used to assess differences in nutrient intakes and BMIs among the intervention groups. ANCOVA (adjusted for baseline values) was used to assess differences among intervention groups in biomarkers at the end of the intervention. Post hoc comparisons between the groups were carried out with Bonferroni corrections. In addition, the use of hormonal medication, BMI, age, sex, prior vitamin D supplement use, and eGFR were tested as covariates, but did not change the significance of the results. We excluded 2 subjects allocated to the plant group from the biomarker analyses due to severe hyperparathyroidism. Data on CTX were not available for 2 subjects (allocated to the animal and 50/50 groups). As a significant interaction between the study group and previous vitamin D supplement use was found in the analyses regarding P-iPTH, the analyses were carried out separately among supplement users (total, $n = 72$; animal group, $n = 26$; 50/50 group, $n = 22$; plant group, $n = 24$) and non-users (total, $n = 62$; animal group, $n = 20$; 50/50 group, $n = 24$; plant group, $n = 18$). Nutrient intake analyses were also carried out separately for those groups. The intakes between users and non-users within each intervention group were compared with independent-samples *t*-tests. All biomarker analyses were also carried out separately for a subpopulation ($n = 29$) having eGFR below the age-specific reference limit for normal kidney function at the endpoint, as well as when these subjects were excluded. No effect of exclusion was observed, and no variables were significant only in that subpopulation. All subjects had eGFRs >60 mL/(min · 1.73 m^2) at the endpoint of the study. Changes over time within each intervention group were tested by paired-samples *t*-tests. Correlations between primary and secondary outcomes were analyzed by Spearman correlations.

Results

Participant characteristics

Background characteristics of the participants are described in Table 1. Altogether, 136 of 145 randomized participants (94%) completed the study. Four-fifths of these were women. No information on menopausal status was available, but 48% of women were at or above the average menopause age of 51 y (24). Participants did moderate-intensity physical activity 3 times per week on average (data not shown). Most of the participants (70%) had at least a secondary-level education (data not shown).

Effects on bone and mineral metabolism: primary outcome

Figure 2 shows the effects of the intervention on biomarkers in blood. S-CTX was significantly higher in the plant group than in the other groups ($P < 0.001$), and differed also between the 50/50 and animal groups ($P = 0.018$). S-iPINP was significantly higher in the plant group than in the animal group ($P = 0.006$). The PINP/CTX ratio was significantly lower in the plant group

TABLE 1 Baseline characteristics of adults that consumed diets differing in animal and plant protein levels for 12 wk

	Animal Diet $n = 46$	50/50 Diet $n = 46$	Plant Diet $n = 44$	All $n = 136$
Women, %	80.4	78.3	77.2	78.7
Age, y	47.6 ± 14.5	47.2 ± 14.7	48.7 ± 14.0	47.8 ± 14.3
Previous[1] vitamin D or multivitamin supplement users, %	56.5	47.8	59.1	54.4
Hormonal medication users (of women), %	54.0	27.8	32.4	38.3
Estimated glomerular filtration rate, mL · min^{-1} · 1.73 m^{-2}	90.0 ± 15.1	90.7 ± 15.1	90.1 ± 13.7	90.3 ± 14.6

Values are means ± SDs for continuous variables and percentages for dichotomous variables. The animal diet contained 70% animal-based and 30% plant-based protein sources of total protein intake; the 50/50 diet contained 50% animal-based and 50% plant-based protein sources of total protein intake; and the plant diet contained 70% plant-based and 30% animal-based protein sources of total protein intake.

[1] Supplement use was advised to be discontinued 2 wk prior to the intervention.

than in the other groups ($P \leq 0.002$). S-CTX was significantly higher in the 50/50 and plant groups ($P \leq 0.034$), and S-iPINP was significantly higher in the plant group ($P < 0.001$), compared with baseline.

At baseline, 83% of participants had adequate vitamin D status (≥50 nmol/L) (27), but the proportion decreased to 60% at the endpoint (among the groups, $P = 0.85$). The mean P-25(OH)D concentration was adequate (27) at baseline in all groups, and the concentrations were significantly higher among previous vitamin D supplement users than among nonusers (72.8 vs. 60.9 nmol/L, respectively; $P = 0.003$). A significant decrease in P-25(OH)D was observed in all groups during the intervention period ($P < 0.001$). In parallel, P-iPTH increased in all groups ($P \leq 0.005$). At the endpoint, among participants who had no history of supplement use, P-iPTH was significantly higher in the plant than in the animal group ($P = 0.018$), but among the subgroup with a history of supplement use, no differences emerged ($P = 0.46$). However, in the subgroup analyses, no endpoint differences in vitamin D status were seen (P values = 0.31–0.72). No differences in S-Ca, S-Pi, P-25(OH)D, or P-iFGF23 were found among the groups (P values = 0.42–0.84).

Figure 3 shows the effects of the intervention on urinary biomarkers. U-Pi excretion was significantly higher in the

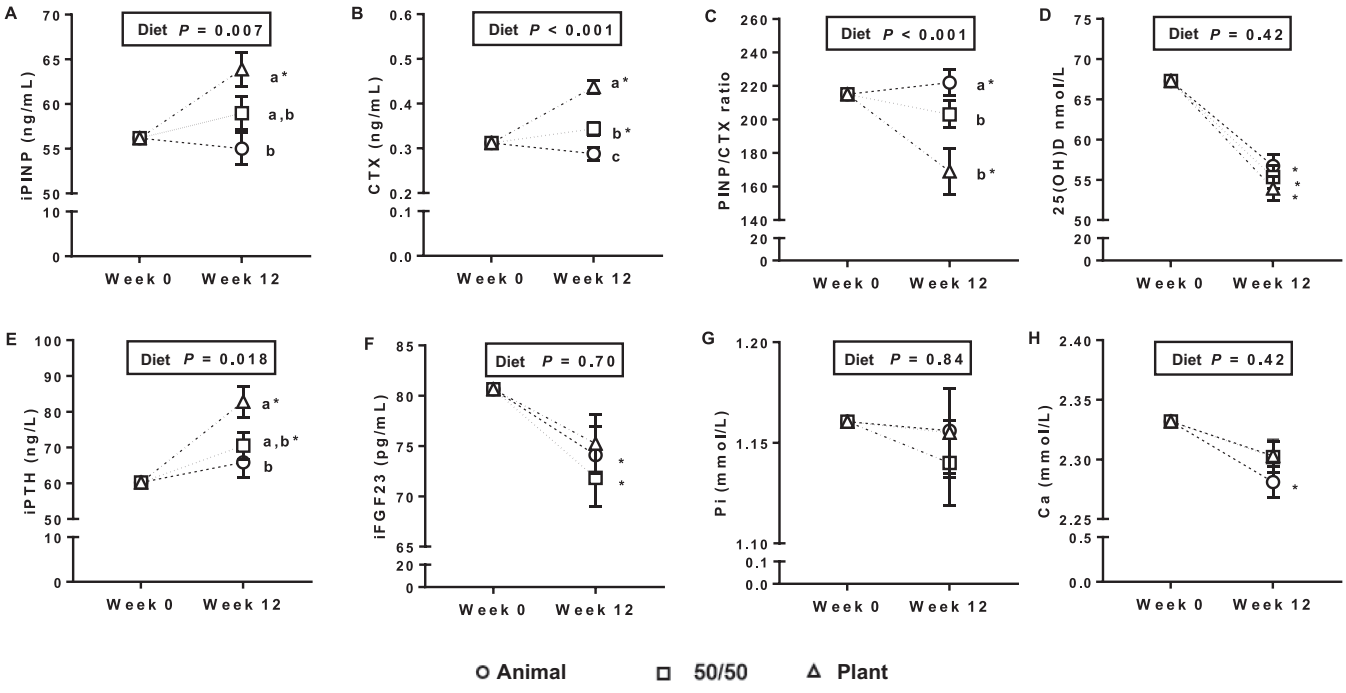

FIGURE 2 Circulating (A) iPINP, (B) CTX, (C) PINP/CTX ratio, (D) 25(OH)D, (E) iPTH (results of participants without previous history of supplement use), (F) iFGF23, (G) Pi, and (H) Ca concentrations of adults that consumed diets differing in animal and plant protein levels for 12 wk. P values are from ANCOVA, adjusted for baseline (Week 0). Bonferroni correction was used for post hoc comparison. Labeled means without a common letter differ at $P < 0.05$. *Indicates a difference from Week 0 at $P < 0.05$ (paired-samples t-test). The animal diet contained 70% animal-based and 30% plant-based protein sources of total protein intake ($n = 46$; CTX and PINP/CTX ratio, $n = 45$; iPTH, $n = 20$); the 50/50 diet contained 50% animal-based and 50% plant-based protein sources of total protein intake ($n = 46$; CTX and PINP/CTX ratio, $n = 45$; iPTH, $n = 20$); and the plant diet contained 70% plant-based and 30% animal-based protein sources of total protein intake ($n = 42$; iPTH, $n = 18$). Abbreviations: Ca, calcium; CTX, collagen type 1 cross-linked C-terminal telopeptide; iFGF23, intact fibroblast growth factor 23; iPINP, intact pro-collagen type I amino-terminal propeptide; iPTH, intact parathyroid hormone; Pi, phosphate; 25(OH)D, 25-hydroxyvitamin D.

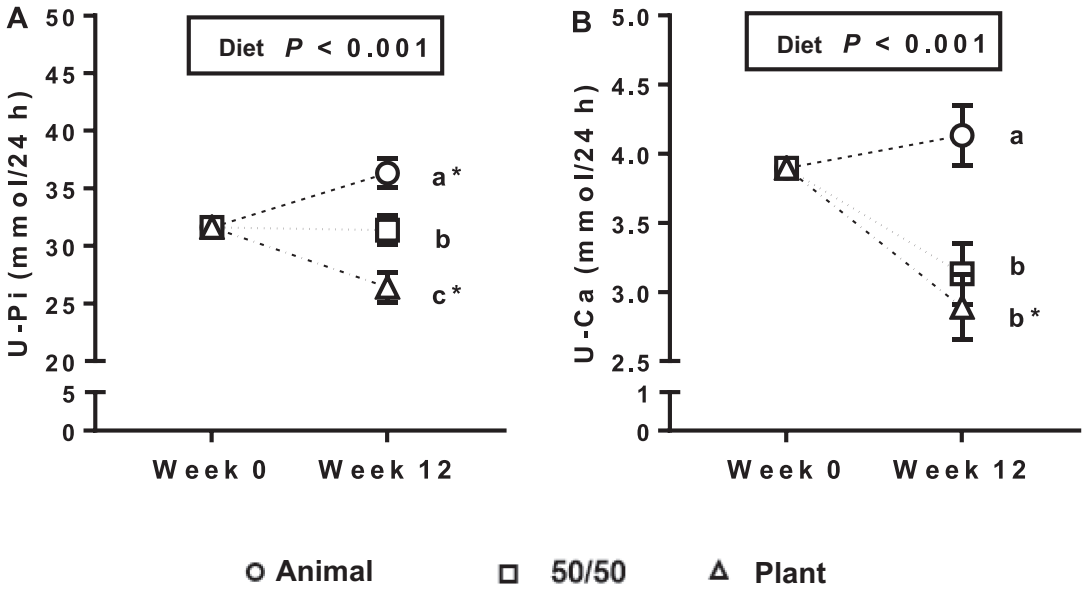

FIGURE 3 The 24-h (A) U-Pi and (B) U-Ca excretion of adults that consumed diets differing in animal and plant protein levels for 12 wk. *P* values are from ANCOVA, adjusted for baseline (Week 0). Bonferroni correction was used for post hoc comparison. Labeled means without a common letter differ at $P < 0.05$. *Indicates a difference from Week 0 at $P < 0.05$ (paired-samples *t*-test). The animal diet contained 70% animal-based and 30% plant-based protein sources of total protein intake ($n = 46$); the 50/50 diet contained 50% animal-based and 50% plant-based protein sources of total protein intake ($n = 46$); and the plant diet contained 70% plant-based and 30% animal-based protein sources of total protein intake ($n = 42$). Abbreviations: U-Ca, urinary calcium; U-Pi, urinary phosphate.

animal group and significantly lower in the plant group than in the other groups ($P \leq 0.025$). U-Ca excretion was also significantly higher in the animal group than in the other groups ($P \leq 0.048$). U-Pi increased in the animal group and decreased in the plant group significantly ($P \leq 0.005$), and U-Ca decreased significantly in the plant group ($P < 0.001$), compared with baseline.

Effects on nutrient intakes: secondary outcome

Table 2 describes nutrient intakes and BMIs in the intervention groups. The protein intake was designed to be 17 E%, but it was significantly lower in the plant group than in the other groups ($P \leq 0.002$). No differences in BMI or in the intakes of energy, carbohydrates (E%), or fats (E%) among the groups were observed (*P* values = 0.09–0.69). Results regarding the intakes of energy-yielding nutrients in grams, as well as percentages of animal and plant proteins in relation to total protein intake, were in line with E% intakes and the designed proportions (70/30%; 50/50%; 30/70%) of animal- and plant-based proteins reported previously (22).

Dietary phosphorus and calcium intakes and molar calcium-phosphorus ratios were significantly higher in the animal group than in the other groups ($P \leq 0.033$), whereas vitamin D intakes differed significantly only between the animal and plant groups ($P = 0.031$). Mean phosphorus intakes in all groups exceeded nutritional recommendations (600 mg/d) by 2- to 3-fold (28). Adequate mean calcium intake (800 mg/d) was reached in the animal and 50/50 groups, but not in the plant group, while the intakes were adequate in all groups at baseline (28). Molar calcium-phosphorus ratios in the 50/50 and plant groups at the endpoint were <0.50, which is far below the recommendation (1:1) for optimal bone health (29). The mean dietary vitamin D intakes were already below the recommended 10 μg/d in all groups prior to intervention (28). At the endpoint, vitamin D intakes in the 50/50 and plant groups did not even reach the average requirement of 7.5 μg/d (28). Correlations between nutrient intakes and selected primary outcomes at the endpoint of the study are shown in **Supplemental Table 2**, and nutrient-nutrient intercorrelations are presented in **Supplemental Table 3**.

Supplemental Table 4 displays the main sources of dietary vitamin D, calcium, and phosphorus. Especially when moving towards more plant-based diets, the role of dairy products as a source of all 3 nutrients decreased, and vegetables and vegetable dishes, as well as cereals and bakery products, became more important sources. For phosphorus intake, nuts and seeds were also significant sources, particularly in the plant group.

Discussion

In the 12-wk clinical intervention study, we investigated the effects of partial replacement of animal protein sources with plant protein sources on markers of bone and mineral metabolism among healthy individuals. We found increased circulating concentrations of PTH, PINP, and CTX and a decreased ratio of bone formation to bone resorption and U-Ca and U-Pi excretion when moving from mostly animal-based protein sources towards plant-based protein sources, indicating that the dietary change leads to accelerated bone turnover. Interestingly, the effects on PTH were only observed in a subgroup that had no history of vitamin D supplement use. Dietary intakes of calcium and vitamin D were below the recommended levels in the plant group. Similar trends in lower intakes of calcium and vitamin D were also observed in the 50/50 group, compared with the animal group. Nevertheless,

TABLE 2 BMI and energy and nutrient intakes of adults that consumed diets differing in animal and plant protein levels for 12 wk

	Animal Diet $n = 46$[1]	50/50 Diet $n = 46$	Plant Diet $n = 44$[2]	*P* for Diet
BMI, kg/m^2				
Week 0	24.7 ± 4.13	24.4 ± 3.92	25.2 ± 3.77	
Week 12	25.0 ± 4.25*	24.5 ± 4.07	25.2 ± 3.73	0.69
Energy, kJ/d				
Week 0	9120 ± 2180	8730 ± 1960	8790 ± 1680	
Week 12	9220 ± 1950	8650 ± 1840	9100 ± 1430	0.20
Protein, E%				
Week 0	18.5 ± 3.23	17.3 ± 3.13	17.7 ± 3.36	
Week 12	18.2 ± 3.10^{a}	16.9 ± 2.15^{a}	15.2 ± 1.98^{b}	<0.001
Carbohydrates, E%				
Week 0	39.0 ± 6.57	40.3 ± 5.82	40.7 ± 5.74	
Week 12	39.8 ± 5.66	41.0 ± 4.55	42.2 ± 5.22	0.09
Fat, E%				
Week 0	37.7 ± 5.70	37.2 ± 6.06	36.6 ± 5.23	
Week 12	36.9 ± 4.89	37.1 ± 5.83	37.1 ± 5.10	0.38
Vitamin D, μg/d				
Week 0	9.8 ± 4.9	8.4 ± 4.2	9.4 ± 5.4	
Week 12	8.2 ± 4.1^{a*}	6.2 ± 3.8^{a,b*}	6.2 ± 3.7^{b*}	0.017
Calcium, mg/d				
Week 0	1300 ± 412	1150 ± 407	1130 ± 292	
Week 12	1180 ± 318^{a}	823 ± 248^{b*}	733 ± 164^{b*}	<0.001
Phosphorus, mg/d				
Week 0	1820 ± 461	1670 ± 444	1660 ± 404	
Week 12	1790 ± 496^{a}	1570 ± 334^{b*}	1540 ± 315^{b}	0.008
Calcium-to-phosphorus ratio, mol/mol				
Week 0	0.55 ± 0.09	0.53 ± 0.09	0.53 ± 0.07	
Week 12	0.51 ± 0.08^{a}	0.40 ± 0.08^{b*}	0.37 ± 0.08^{b*}	<0.001

Values are means ± SDs. *P* values (for diet) are from one-way ANOVA. Bonferroni correction was used for post hoc comparison. Labeled means in a row without a common letter differ at $P < 0.05$. *Indicates a difference from Week 0 at $P < 0.05$ (paired-samples *t*-test). The animal diet contained 70% animal-based and 30% plant-based protein sources of total protein intake; the 50/50 diet contained 50% animal-based and 50% plant-based protein sources of total protein intake; and the plant diet contained 70% plant-based and 30% animal-based protein sources of total protein intake.

[1] For baseline (Week 0) dietary data, $n = 45$.

[2] For baseline (Week 0) dietary data, $n = 43$.

no differences emerged in vitamin D status between the groups, although 25(OH)D concentrations decreased in all study groups during the intervention.

Adequate protein intake is important for bone health (8), but previous systematic reviews and meta-analyses have not found differences between animal and plant proteins in their effects on bone mineral density (10, 17, 18). However, the plant proteins used in randomized controlled trials have mainly been soy protein isolates, and the results cannot be generalized to other plant protein sources. Our plant-based protein sources, in addition to soy-based tofu, were cereal products (including oats, rye, wheat, and barley), fava beans, peas, and seeds and nuts. Protein intake [E% and g/d (22)] was slightly but significantly lower in the plant group than in the other groups, and a similar trend was observed in urinary protein excretion (22). Nonetheless, the intake of protein (15.2 E%) was in accordance with recommendations [10–20 E%, (28)]; thus, this is not a case of low protein intake, which has been shown to be detrimental for bone health (18). However, plant-based proteins can have lower bioavailability than animal-based proteins (30). Animal protein intake in our study correlated negatively with CTX and PINP, whereas a positive correlation emerged between plant protein intake and CTX. Additionally, in the plant group, both turnover markers correlated negatively with total protein intake (E%). Nevertheless, it is not clear whether the differences in protein intakes or quality play a major role in the effects on bone turnover, because we also observed a strong intercorrelation between the nutrients examined. One could speculate that different amino acid compositions of the diets may have an effect on bone turnover. Arginine and lysine have been shown to stimulate bone health in various ways (11–13, 15), but arginine is generally found in both animal- and plant-based sources. Lysine can be the limiting amino acid if a plant-based diet is comprised solely of cereal protein (16); however, the plant group consumed other sources of plant-based proteins in addition to cereal protein, not forgetting the 30% animal-based proteins. As amino acid compositions of the study diets were not available, it is impossible to determine whether the effects on bone turnover were related to specific amino acids.

We could assume that the increased bone turnover in the diets containing more plant-based proteins is mainly a result of a combination of decreased calcium and vitamin D intakes and a lower calcium-phosphorus ratio, which has previously been shown to be harmful for bone health (29, 31). Moreover, phytic acid, abundant in the plant-protein foods but rarely reported in the food composition databases, can interfere with calcium absorption, contributing to lower calcium bioavailability from the plant-protein diets (32). Supporting this view, U-Ca excretion was also lower in the 50/50 and plant groups. However, differences in PTH among the groups were seen only among those individuals who had not previously used vitamin D supplements. They had lower

vitamin D status already at baseline than their peers with a history of supplement use. Interestingly, when the dietary groups were analyzed separately and stratified by previous supplement use, nonusers in the plant group had lower calcium intake than former users at the endpoint of the study. Lower calcium intake in this subgroup (**Supplemental Table 5**) may have contributed to higher endpoint PTH concentrations, since calcium prevents increases in PTH (32). The active form of vitamin D, 1,25-dihydroxyvitamin D, decreases both PTH and FGF23 production (33). Therefore, the lower baseline 25(OH)D concentration, in combination with low vitamin D intake during the intervention among previous supplement nonusers, may have led to lower concentrations of the active metabolite and also, in combination with low calcium intake, to higher PTH, although no differences in FGF23 were observed between any of the groups. However, based on our study design and results, we cannot conclude whether the effects of the 12-wk plant diet on bone and mineral metabolism are transient. The bone remodeling transient—that is, the temporary alteration in the equilibrium between bone formation and resorption, in this case caused by the diet—is 3 mo for young adults and 6–18 mo for older adults (34). Our participants were aged 20–69 y; thus, some of them may have reached a new balance in bone turnover during the study period. A longer follow-up and measurements after the intervention would elucidate this point.

The decline in vitamin D status in all intervention groups was obvious due to minimal or nonexisting sunlight during most of the study period. More than half of the participants had previously used vitamin D supplements and were advised to discontinue their use for only 2 wk prior to the intervention. This was not sufficiently long to exclude the effects of supplementation on vitamin D status, because the half-life of 25(OH)D is 15 d (35). As vitamin D–fortified fluid milk products are generally an important source of vitamin D in Finland (24), their restricted intake most likely caused a decrease in vitamin D intake. Nonfortified plant-based drinks were delivered to the groups, in which animal proteins were replaced with plant proteins. However, these drinks are not equivalent to cow's milk, nor are they sources of protein, calcium, or vitamin D if not fortified (36). Thus, when promoting more sustainable plant-based diets and simultaneously ensuring adequate vitamin D and calcium intakes, it is important to include in the diet either foods that are natural sources of these nutrients or that have been fortified either with vitamin D and calcium or with supplements containing these nutrients.

We did not observe any effects of the diets on FGF23 and S-Pi concentrations; however, U-Pi excretion and dietary phosphorus intake were higher in the 50/50 and plant groups than in the animal group. This is in line with the results of a previous 4-wk study among kidney patients on a diet containing 70% plant-based (of total) protein, where U-Pi excretion decreased, but no differences were observed in FGF23, PTH, or S-Pi (21). In 2 cross-over studies with 70% and 95% animal-based proteins for 7 wk and for 1 wk, there were higher S-Pi concentrations; in the latter study, higher FGF23 and lower PTH concentrations were also observed in the animal-based protein diets than in diets high in plant-based protein (19, 20). However, results of studies carried out among subjects with impaired renal function are not fully comparable with our results in a healthy population. One explanation for finding no differences in blood phosphate metabolism markers in our study is that in the animal protein–based group, phosphorus intake was comparable to that at baseline (that is, not very high), thus being attainable through a regular diet.

Strengths of the study are a comprehensive study population with a high compliance rate; there were only 9 drop-outs, while 136 subjects completed the study. The bone turnover markers used were the ones recommended by the International Osteoporosis Foundation (37). The duration of the study was 12 wk, which is longer than usual for dietary interventions. However, regarding bone remodeling, a longer duration would have been preferable, but compliance might have suffered. The study participants had higher education than Finns in general; thus, they may have been more health-conscious, which could have hidden some effects of the study due to more healthy diets already at baseline. Had vitamin D and multivitamin supplements been advised to be discontinued earlier than 2 wk prior to the study, this would have yielded more trustworthy results on the effects of diets on 25(OH)D. One weakness of the study was that physical activity was only measured using a questionnaire instead of an accelerometer, which would have been more accurate.

To conclude, partial replacement of animal proteins with plant-based protein sources increased markers of bone resorption and formation among healthy individuals during the 12-wk controlled clinical intervention, indicating a risk for bone health. Low calcium and vitamin D intakes, as well as a low calcium-to-phosphorus ratio in the diets containing more plant-based protein sources, may have contributed to accelerated bone turnover, but it remains unclear whether differences in protein intake or quality play a major role. To maintain bone health, it is important to ensure adequate intakes of vitamin D and calcium when recommending that populations increase their consumption of more sustainable, plant-based foods.

Acknowledgments

We thank the food companies Apetit Plc, Arola Farm, Atria Plc, Fazer Ltd, Gold&Green Foods Ltd, HKScan Plc, Jalofoods/Oy Soya Ltd, Lantmännen Unibake International Ltd, Mauste-Sallinen Ltd, Meira Ltd, and Verso Food Ltd for providing foodstuffs for the intervention. The volunteers of ScenoProt intervention study are warmly thanked for their contributions.

The authors' responsibilities were as follows—STI, A-MP, and EP: designed the research; A-MP, EP, and TP: conducted the research; JR: provided the essential reagents; HV and TP: analyzed the dietary data; STI: performed the statistical analysis, wrote the manuscript draft, and had primary responsibility for the final content; STI, A-MP, EP, ME, and CL-A: worked on the manuscript; and all authors: read and approved the final manuscript.

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