Dietary phosphorus intake is negatively associated with bone formation among females and positively associated with some bone traits among males – a cross-sectional study in middle-aged Caucasians

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Abbreviations

25(OH)D; 25-hydroxyvitamin D

aBMD; areal bone mineral density

BMC; bone mineral content

BMD; bone mineral density

BMI; body mass index

Ca; calcium

CTX; carboxy-terminal collagen crosslink

CV; coefficient of variation

iPINP; intact pro-collagen type I amino-terminal propeptide

P; phosphorus

PTH; parathyroid hormone

SE; standard error

vBMD; volumetric bone mineral density

Abstract

High dietary phosphorus (P) intake has acute negative effects on calcium (Ca) and bone metabolism, but long-term clinical data are contradictory. We hypothesized that high P intake is associated with impaired bone health as suggested by earlier short-term studies on bone metabolism. In this cross-sectional study, we investigated associations between dietary P intake, bone traits in the radius and tibia, and bone turnover in a population-based sample of 37- to 47-year-old Caucasian premenopausal females (n=333) and males (n=179) living in Southern Finland (60ºN). We used various regression models in an "elaboration approach" to elucidate the role of P intake in bone traits and turnover. The addition of relevant covariates to the models mainly removed the significance of P intake as a determinant of bone traits. In the final regression model (P intake, weight, height, age, Ca intake, serum 25-hydroxyvitamin D, physical activity, smoking, contraceptive use in females), P intake was slightly positively associated only with bone mineral content and cross-sectional cortical bone area in the tibia of males. Among females, inclusion of Ca removed all existing significance in the crude models for any bone trait. In females P intake was negatively associated with the bone formation marker serum intact pro-collagen type I amino-terminal propeptide, while no association was present between P intake and bone turnover in males. In conclusion, these findings disagree with the hypothesis; P intake was not deleteriously associated with bone traits, however, P intake may negatively contribute to bone formation among females.

Keywords: phosphorus, calcium, bone mineral density, bone turnover markers, peripheral quantitative computed tomography, cross-sectional study

1. Introduction

An estimated 15% of adults aged >50 years in Finland suffer from osteoporosis [1]. However, during this millennium femoral neck bone mineral density (BMD) among elderly Finnish women has been observed to increase, indicating improved bone health in this group [2]. Due to frequent dairy product consumption, calcium (Ca) intake has generally been adequate among Finns [3]. Also vitamin D intake has increased over the last decade due to the food fortification policy [3,4]. Besides Ca, phosphorus (P) is known to be essential for bone tissue as a form of hydroxyapatite [5]. Nevertheless, there is concern about potential detrimental effects of excess dietary P intake on bone health due to a potential imbalance in Ca-P homeostasis. Short-term experimental studies in humans have shown that excess P intake together with low Ca intake is detrimentally associated with calcium and bone metabolism in terms of e.g. augmented serum parathyroid hormone (PTH) concentrations and/or impaired bone turnover [6-9]. In animals, long-term high P intake with a normal Ca intake has been shown to cause bone loss and impaired bone mineralization [10,11]. In contrast, clinical human data on the relationship between P intake and BMD and bone mineral content (BMC) are contradictory, and some studies are confounded by adequate Ca intake and small number of subjects [12-18]. Concerns about high P intake and bone are relevant because P intake in Western countries exceeds 2- to 3-fold the nutritional recommendations (600-700 mg/d) [3,19-22]. Especially the increased use of food additive phosphates has augmented P intake; up to 50 % of P intake may originate from additives [23], which have been suggested to be more harmful for bone metabolism than natural P [24-28].

Considering the possible deleterious role of high P intake in bone metabolism, we hypothesized that dietary P intake is negatively associated with bone health in terms of decreased bone formation and increased resorption as well as decreased bone size, BMC, and BMD. We used an

96 "elaboration approach" to elucidate the role of P intake in radial and tibial bone structure
97 measured by peripheral quantitative computed tomography (pQCT) and bone turnover in 37- to
98 47-year-old Caucasian adults in a cross-sectional design, taking into account other important
99 *intrinsic* and *extrinsic* factors in bone health.
100
101 **2. Methods and materials**
102 **2.1 Subjects**
103
104 The population-based study carried out in January-May 2010 comprised recruitment of 37- to
105 47-year-old Caucasian females and males in the Helsinki area (60ºN). Recruitment of the
106 participants and the study protocol are described in detail elsewhere [29]. Pregnant women were
107 excluded from the study. The total number of recruited participants for the first phase of the
108 study was 678. Of these, 653 participated in the second phase where bone measurements were
109 carried out. In the final analysis of this substudy, 141 participants were not included due to
110 incomplete data or exclusion criteria (menopause, earlier history of eating disorder, medication
111 affecting Ca or bone metabolism, or moderate renal dysfunction, i.e. estimated glomerulus
112 filtration rate (eGFR) < 60 mL/min) [30], resulting in 179 males and 333 premenopausal
113 females, for whom full nutrition, pQCT, background, and biomarker data were available. This
114 study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all
115 procedures involving human subjects were approved by the Helsinki Uusimaa Hospital District
116 Ethics Committees. Written informed consent was obtained from all participants.
117
118 **2.2 Bone assessments**
119 Distal (4% and 5% site) and shaft sites (30%) of the radius and tibia were measured with pQCT
120 (XCT 2000R, Stratec Medizintechnik Gmbh, Pforzheim, Germany). The radius of the non-

dominant side and the left tibia were scanned, except for subjects who had previous fractures or metal implants in the scan site; their contralateral site was measured. The pQCT scanning and the analysis protocol, earlier used in the GENDI Study [31], were based on well-established protocols [32]. Total BMC (mg), total bone cross-sectional area (mm^2), cortical bone area (mm^2), trabecular bone density (mg/cm^3), and cortical bone density (mg/cm^3) were assessed. *In vivo* coefficients of variation (CV%) for the radius were 2.5% for distal total area and 3.9% for shaft site, 4.4% for cortical area at distal site and 1.1% at shaft site, 1.6% for trabecular density at distal site, and 0.5% for cortical density at shaft site. For the tibia, the corresponding values were 1.3%, 1.2%, 2.6%, 1.2%, 0.5%, and 0.6%. The long-term stability of the scanner was assessed by daily phantom scans, which showed constant density levels over the study period.

2.3 Dietary intake data

Habitual dietary intake data of subjects were collected by 3-day food records, which included two weekdays and one weekend day. The subjects were instructed to maintain their normal food habits during the recording period and to record all foods, beverages, and dietary supplements immediately after consumption. Nutrient intake was calculated using a computer-based program (Diet 32 version 1.4.6.2, Aivo2000, Turku, Finland), which is obtained from the Finnish food composition database Fineli®, developed and continuously updated by the Finnish National Institution of Health and Welfare (www.fineli.fi). The background data questionnaire included a question about the use of vitamin and mineral supplements, allowing more accurate calculations of total Ca and vitamin D intakes.

2.4 Bone biomarkers

Twelve-hour fasting blood samples were collected between 7:30 am and 9:15 am. Serum was extracted from blood by centrifugation and stored immediately after sampling at -20°C or -70°C until analysis. Serum phosphate (S-Pi), serum creatinine (S-Krea), serum 25-hydroxyvitamin D (S-25(OH)D), and serum PTH (S-PTH) concentrations were analyzed at the Department of Food and Environmental Sciences, University of Helsinki, in 2010. S-Pi was analyzed by a spectrophotometric molybdate method using a Konelab20 automatic analyzer (Thermo Clinical Labsystems Oy, Espoo, Finland) [33]. S-Krea was analyzed by Jaffe method using Konelab20 [34]. Inter- and intra-assay coefficients of variation (CV%) for S-Pi and S-Krea were <4.6%. S-25(OH)D concentrations were analyzed by enzyme-immuno assay with IDS EIA Kit (Immunodiagnostics Systems Ltd., Bolton, UK) [35]. Inter- and intra-assay CV% were 2.7% and 3.2%, respectively, based on the provided controls measured in the laboratory. At the time that the samples were analyzed, the laboratory was in the process of achieving the Vitamin D External Quality Assessment Scheme certificate, DEQAS (deqas.kpmd.co.uk/), for ensuring reproducibility of analyses. The laboratory received the DEQAS proficiency certificate for this method in 2012. S-PTH concentrations were analyzed by a two-site chemiluminescent enzyme-labeled immunometric assay by Immulite1000 (Siemens Healthcare Diagnostics, NY, USA) [36]. Inter- and intra-assay CV% were for the low control sample 7.6% and 1.0%, and for the high control sample 7.9% and 5.4%, respectively. Serum intact pro-collagen type I amino-terminal propeptide (S-iPINP) and serum collagen type 1 cross-linked C-terminal telopeptide (S-CTX) were analyzed by chemiluminescence immunoassay using an IDS-iSYS Multidiscipline Automated Analyzer (Immunodiagnostic Systems Ltd., Bolton, UK) at the NordLab Oulu, and at the Department of Clinical Chemistry of the University of Oulu in 2012 [37,38]. For both assays, intra CV% was <5.3% and inter CV% <2.9%.

2.5 Background data collection

Data on background variables (such as physical activity, disease history and medication, smoking, menopausal status) were collected by a self-administered questionnaire that included completion instructions. The questionnaire was checked by researchers at the research unit, and lacking information was requested if needed. Smoking was classified as current/former smoker or never-smoker. In the present analyses of females, only those reporting regular menstruation were included. Physical activity was expressed as a frequency, and duration of exercise or exercise training was calculated as min/week. Body mass index (BMI, kg/m^2) was calculated based on height and weight measured in light clothing with a standard stadiometer (to the nearest half cm) and scale (to the nearest 100 g) at the research unit.

2.6 Statistical analyses

The final number of participants was intended to be 800 persons (400 females, 400 males). The sample size is based on a statistical power of 80% (α=0.05) to find a 4% difference (standard deviation; SD=0.050 g/cm^3) in distal radius trabecular density between the highest and lowest P intake tertiles by analysis of variance, and takes into account an initial drop-out of 40% (based on 1200 subjects).

Statistical analysis was performed using SPSS Statistics version 21 (IBM, Armonk, NY, USA). The normality and homogeneity of the data were verified and log-transformed to improve normality if needed. Statistically significant data was determined for a P value less than 0.05. Data are shown as means and standard deviations. Differences between sexes concerning background variables were analyzed by the Mann-Whitney U-test. Associations between P intake, pQCT data, bone turnover markers, and potential covariates were assessed by Spearman

196 correlation coefficients (data not shown). After the initial observation that P intake was not a
197 strong determinant of bone traits, whereas it was very sensitive to other relevant explanatory
198 variables in the regression model, we chose to use the so-called elaboration approach [39].
199
200 To evaluate the role of P intake in bone health, we used the elaboration technique, which has
201 been widely used in social sciences to understand the composite effect and the dependency
202 structure of several determinants [39]. Our approach is modified as it sets the focus on the effect
203 magnitudes instead of partial correlations. Thus, we compared the resulting regression
204 coefficients among several models [39]. In the elaboration approach, the best-fit model is not
205 sought; rather the aim is to observe and compare the effect of P intake on bone characteristics
206 across various models, i.e. in different contexts determined by other factors known to modulate
207 the bone characteristics. The rationale of this approach is to regard multicollinearity more as a
208 source of information than as a nuisance. Elaboration looks for the interplay between the
209 different explanatory variables on the importance of P intake.
210
211 Hence, the data were analyzed using several regression models with different combinations of
212 determinants (explanatory variables) of bone traits/turnover. The starting model always had P
213 intake as the only explanatory variable. After this, the other relevant explanatory variables were
214 added to the model and the resulting beta coefficients, i.e. slopes, of P intake were compared.
215 Based on changes in the slopes, the role of P intake among bone variables was interpreted. We
216 chose to study the following determinants of bone traits/turnover mainly for their contextual
217 relevance based on earlier literature: weight, height, age, Ca intake, contraceptive use among
218 females, smoking, S-25(OH)D, and physical activity [40-42]. The logic of inclusion order was to
219 start with variables that have an established, '*intrinsic*' effect on bone characteristics, i.e. height,
220 weight, and age. After this, *smaller scale, 'extrinsic'* variables were added (first: Ca intake, then:

contraceptive use (not in males), smoking, S-25(OH)D concentration, and physical activity). This logic resulted in five different models (Table 1). We also created models with S-PTH, but as its effect was negligible, the results of these models are not reported.

3. Results

Background, nutrient intake, and biochemical data of the study subjects are shown in Table 2. Beta coefficients, i.e. slopes, of P intake in the regression models are presented in Table 3. In each bone variable, the model with the highest adjusted r^2 is indicated in boldface in Table 3 (adjusted coefficients of determination are given in Table 4). In the results section, we have concentrated on those variables where relevant changes in coefficients (i.e. slopes) of P intake were observed. Cortical and trabecular bone mineral densities are only reported in the tables because P intake was not significant in any model of cortical or trabecular density for either sex. Bone measurements (BMC and BMD) and traits are presented in Table 5.

3.1 Bone turnover markers

Adjusted R^2s in the different bone turnover marker models ranged from 0.020 to 0.091 among females and from 0.000 to 0.096 among males (Table 4). Among females, the slope of P intake was negative in all models of S-iPINP (for statistical significances, see Table 3), i.e. P intake was a negative determinant of bone formation. After adding Ca (Model 4), the slope of P intake became more negative and remained significant. In the models of S-CTX, P intake was a significantly negative determinant until Ca intake was introduced to the model. However, concerning both S-iPINP and S-CTX, adjusted R^2 increased significantly only after introducing weight and height to the model. Among males, P intake was not a significant determinant of bone turnover markers, nor did the additional determinants affect the slope.

3.2 Bone mineral content

Adjusted R^2s in the models of BMC ranged from 0.025 to 0.328 among females and from 0.004 to 0.253 among males (Table 4). P intake was a significant positive determinant of distal tibia (females and males), tibial shaft (females), and distal radius (females) until Ca intake was added to the model. The role of P attenuated when other explanatory variables were introduced to the model. Significance of P intake as a positive determinant of tibial shaft BMC was present in all models among males. Introducing height and weight attenuated the association; the inclusion of Ca, however, reversed this effect. In radial shaft BMC, P intake was a significant positive determinant only in Model 1 among females, and no significance was found among males. An increase in the total adjusted R^2s was observed when height and weight were introduced to the models among both sexes.

3.3 Cross-sectional total and cortical bone area

Adjusted R^2s in the models of cross-sectional bone area ranged from 0.004 to 0.356 among females and from 0.000 to 0.326 among males (Table 4). P intake was a positive determinant of total bone area among females until height and weight were introduced to the model, after which the significances weakened. Among both sexes, P intake was a significant positive determinant of total bone area of distal tibia until Ca was added to the model. In total bone area of radial shaft, also among males, the inclusion of weight and height removed the significance of P intake. Further, in cortical bone area of radial and tibial shaft, as well as distal tibia among females, P intake was significant. In distal tibia, this significance was present only in the first model, and in the shaft sites the significance disappeared after adding Ca. Among males, P intake was a significant positive determinant of cortical bone area of distal tibia and tibial shaft in the three first models, and the inclusion of Ca removed the significance. Moreover, P intake was

significant in Model 5 of tibial shaft among males, but not in Model 4. In total bone and cortical area, increases in R^2s occurred after introducing height and weight to the models.

4. Discussion

We examined the association of the effect of P intake on several bone variables in a middle-aged Finnish population of females and males. Of interest were bone traits in the radius and tibia, measured by pQCT, indicating long-term bone health as well as bone turnover markers, reflecting acute bone metabolism. The elaboration approach was used to statistically elucidate the role of P intake, among other various *intrinsic* and *extrinsic* factors, in bone health. With regard to bone turnover markers, significant results were observed only in females. Dietary P intake was negatively associated with S-iPINP, a bone collagen formation marker, possibly indicating impaired bone formation with higher P intake. P intake was also negatively related to S-CTX, indicating decreased collagen degradation with increasing P intake among females. However, when Ca was introduced to the model, the association disappeared. Among males, significant associations between P intake and bone turnover were not observed. Concerning the bone traits, significant results were mainly seen in tibial bone for men.

The results about bone traits were contradictory to both our hypothesis and the evidence from short-term studies on bone metabolism [6-9]; associations between P intake and bone traits were positive. The observed beta coefficients (slopes) of P intake and adjusted coefficients of determination in different regression models indicate that dietary P intake overall is not a strong determinant of BMC, bone cross-sectional area, and cortical or trabecular density. Further, the significance of P intake as a determinant attenuated when Ca intake was introduced to the models, but inclusion of Ca did not improve coefficients of determination, i.e. the explanatory

power of the models. Ca somehow compensated the effect of P intake as a weak determinant in bone traits. Based on the results, the role of P intake as a determinant of bone traits does not seem to be clinically relevant.

Differences emerged in the associations between dietary P and bone traits among the sexes. Ca seemed to be stronger modulator of bone among females than among males. An earlier study showed that some differences exist between the sexes in maintaining Ca homeostasis; women may be more vulnerable to high P intake, especially through Ca and PTH metabolism [43]. Earlier short-term randomized controlled trials carried out on younger women have revealed that high P intake increases S-PTH concentrations when Ca intake is low, and decreases bone formation; this may result in persistently elevated S-PTH concentrations [6-9]. In our cross-sectional study, we observed a similar association with the bone formation in females, but not in males, possibly strengthening the evidence of sex-specific differences in effects of P intake on bone turnover. However, S-PTH did not seem to be a mediator of the effects of P because adding it to the models did not change the results. The cross-sectional design here may be one explanation for not finding an effect of S-PTH.

Earlier data on P intake and bone, excluding bone turnover and Ca metabolism markers, in Western countries are scarce, and due to the dual-energy X-ray absorptiometry (DXA) measurements, comprise only areal BMD (aBMD) and not volumetric BMD data like pQCT [14,15,17,18]. A small cross-sectional study of 24- to 28-year-old females showed a negative association between P intake and radial aBMD [15]. In women aged 18-31 years, dietary P intake was positively correlated with radial aBMD and spine aBMD and BMC [17]. Whiting et al. [18] found out that P intake positively predicted total body and lumbar spine aBMD in middle-aged males, but no effect on hip aBMD was observed. A recent study of the National

Health and Nutrition Examination Survey data showed that high P intake was positively associated with femoral BMC in teenage girls, and with femoral BMC and aBMD in adults [14]. Our new data suggest significant positive associations between P intake and weight-bearing tibial sites, especially among males; however, no association between P intake and volumetric BMD was found. All of these above-mentioned observations were made under circumstances in which Ca intake was at least satisfactory – this confounding effect cannot be excluded. Further, the measured bone sites differ between these studies and the results are conflicting, complicating the drawing of conclusions about the role of P intake in bone health.

The associations between P intake and BMC, bone cross-sectional area, and cortical and trabecular density are influenced by many factors. Our study showed that BMC and bone cross-sectional area are especially related to height but also to some extent to weight, as persons with larger body size have larger bones. Thus, the small but positive association between P intake and BMC and bone cross-sectional area in males may be due to men, as generally taller and larger persons, eating more and thus getting more P from the diet. However, we did not adjust the data for energy intake because we aimed to observe the effect of absolute P intake on bone variables using models that also included other factors known to play a role in bone structure and metabolism. As vitamin D plays a role in bone homeostasis [43], we also have to point out that the fairly satisfactory S-25(OH)D concentrations (mean >50 nmol/L, defined as the cut-off level for sufficient vitamin D status by the Institute of Medicine [41]) among our study participants may have masked the potentially harmful associations between P and bone.

Strengths of our study are the large population-based sample consisting of both females and males (albeit less males than females), assessment of several bone traits with pQCT in two functionally different bones (radius and tibia) and sites (distal site and diaphysis), analysis of

relevant biomarkers, and extensive background data. Our study population is representative of nutrient intakes in the same-aged Finnish FINDIET Study population in the Helsinki area; Ca, P, and energy intakes were similar, also their BMIs were concordant [3]. Moreover, in contrast to commonly used DXA, providing ambiguous areal BMD values [44], pQCT provides relevant data for trabecular and cortical densities as well as bone geometry, size, and mass [32]. All of these traits are relevant to bone strength and may be differently associated with nutrient intakes, as the present results indicated. A limitation of this study is its cross-sectional design; we were unable to take into account the earlier diet of the subjects such as earlier exposure to high P intake. Thus, the associations with bone turnover may be more relevant than the associations with bone traits. We also did not specifically evaluate the confounding influence of bone-loading activity on bone traits or take the history of physical activity into account. Moreover, we did not consider the potential influence of genetic factors [45]. We also did not distinguish P intake from different sources. It would have been interesting to see how food additive phosphates contribute to bone health. The statistical analysis design did not allow the use of scoring technique for food additive phosphorus intake that was utilized in our earlier study on the same population [29].

In conclusion, in the present sample of a middle-aged Finnish population with adequate Ca intake, we found that P intake was generally not a determinant of bone traits measured by pQCT. The weak positive association between P intake and tibial BMC and bone cross-sectional area in males may be due to men, as larger persons, eating more, and thus, getting more P from their diet. However, no associations were observed between P intake and bone turnover among males, while among females P intake was associated with reduced bone turnover. Overall, adding Ca as a determinant to the models seemed to attenuate the association between P intake and bone traits, and this effect was stronger among females. Prospective studies on the association between high

P intake, especially in the form of highly absorbable food additive phosphate, and potential bone deterioration are needed, particularly among people with low Ca intake.

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Conflicts of interest

JR has a patent for the PINP assessment method, but the royalty period has expired. STI, HJR, EMS, VEK, HJK, MUMK, MHP, EKAL, HS, MKK, and CJLA have no conflicts of interest to report.

References

[1] Svedbom A, Hernlund E, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Epidemiology and economic burden of osteoporosis in Finland. Arch Osteoporos 2013;8:137.

[2] Uusi-Rasi K, Karinkanta S, Heinonen A; Sievänen H. Improved femoral neck BMD in older Finnish women between 2002 and 2010. Maturitas 2013;75:241-5.

[3] Helldán A, Raulio S, Kosola M, Tapanainen H, Ovaskainen M-L, Virtanen S. Finravinto 2012 –tutkimus – The National FINDIET 2012 Survey. Reports of Institution of Health and Welfare, 2013_016.

[4] Itkonen ST, Lamberg-Allardt C. Food fortification as a means to increase vitamin D intake. Br J Nutr 2016;115:2075-6.

[5] Penido MG, Alon US. Phosphate homeostasis and its role in bone health. Pediatr Nephrol 2012;27:2019-48.

[6] Calvo MS, Kumar R, Heath III H. Persistently elevated parathyroid hormone secretion and action in young women after ingesting high phosphorus, low calcium diets. J Clin Endocrinol Metab 1990;70:1334-40.

[7] Kärkkäinen M, Lamberg-Allardt C. An acute intake of phosphate increases parathyroid hormone secretion and inhibits bone formation in young women. J Bone Miner Res 1996;11:1905-12.

[8] Kemi VE, Kärkkäinen MUM, Lamberg-Allardt CJE. High phosphorus intakes acutely and affect Ca and bone metabolism in a dose-dependent manner in healthy young females. Br J Nutr 2006;96:545-52.

[9] Kemi VE, Kärkkäinen MU, Karp HJ, Laitinen KA, Lamberg-Allardt CJ. Increased calcium intake does not counteract the effects of increased phosphorus intake on bone: an acute dose-response study in healthy females. Br J Nutr 2008;99:832-9.

[10] Huttunen MM, Pietilä PE, Viljakainen HT, Lamberg-Allardt CJ. Prolonged increase in dietary phosphate alter bone mineralization in adult male rats. J Nutr Biochem 2006;17:479-84.

[11] Huttunen MM, Tillman I, Viljakainen HT, Tuukkanen J, Peng Z, Pekkinen M, et al. High dietary phosphate intake reduces bone strength in the growing rat skeleton. J Bone Miner Res 2007;22:83-92.

[12] Ito S, Ishida H, Uenishi K, Murakami K, Sasaki S. The relationship between habitual dietary phosphorus and calcium intake, and bone mineral density in young Japanese women: a cross-sectional study. Asia Pac J Clin Nutr 2011;20:411-7.

[13] Lee KJ, Kim KS, Kim HN, Seo JA, Song SW. Association between dietary calcium and phosphorus intakes, dietary calcium/phosphorus ratio and bone mass in the Korean population. Nutr J 2014;13:114.

[14] Lee AW, Cho SS. Association between phosphorus intake and bone health in the NHANES population. Nutr J 2015;14:28.

[15] Metz JA, Anderson JJB, Gallagher Jr PN. Intakes of calcium, phosphorus and protein, and physical-activity level are related to radial bone mass in young adult women, Am J Clin Nutr 1993;58:537-42.

[16] Sasaki S, Yanagibori R. Association between current nutrient intakes and bone mineral density at calcaneus in pre- and postmenopausal Japanese women. J Nutr Sci Vitaminol (Tokyo) 2001;47:289-94.

[17] Teegarden D, Lyle RM, McCabe GP, McGabe LD, Proulx WR, Michon K, et al. Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. Am J Clin Nutr 1998;68:749-54.

[18] Whiting SJ, Boyle JL, Thompson A, Mirwald RL, Faulkner RA. Dietary protein, phosphorus and potassium are beneficial to bone mineral density in adult men consuming adequate dietary calcium. J Am Coll Nutr 2002;21:402-9.

[19] Calvo MS. Dietary phosphorus, calcium metabolism and bone. J Nutr. 1993;123:1627-33.

[20] Nordic Council of Ministers. Nordic Nutrition Recommendations 2012. Nord 2013:009.

[21] Uribarri J, Calvo M. Dietary phosphorus excess: A risk factor in chronic bone, kidney, and cardiovascular disease? Adv Nutr 2013;4:542-4.

[22] Welch AA, Fransen H, Jenab M, Boutron-Ruault MC, Tumino R, Agnoli C, et al. Variation in intakes of calcium, phosphorus, magnesium, iron and potassium in 10 countries in the European Prospective Investigation into Cancer and Nutrition study. Eur J Clin Nutr. 2009;63:S101-21.

444 [23] Winger RJ, Uribarri J, Lloyd L. Phosphorus-containing additives: An insidious danger for
445 people with chronic kidney disease. Trends Food Sci Technol 2012;24:92-102.
446 [24] Bell RR, Draper HH, Tzeng DYM, Shin HK, Schmidt GR. Physiological responses of
447 human adults to foods containing phosphate additives. J Nutr 1977;107:42-50.
448 [25] Fernando GR, Martha RM, Evangelina R. Consumption of soft drinks with phosphoric acid
449 as a risk factor for the development of hypocalcemia in postmenopausal women. J Clin
450 Epidemiol 1999;52:1007-10.
451 [26] Karp HJ, Vaihia KP, Kärkkäinen MU, Niemistö MJ, Lamberg-Allardt CJE. Acute effects of
452 different phosphorus sources on calcium and bone metabolism in young women: a whole foods
453 approach. Calcif Tissue Intl 2007;80:251-8.
454 [27] Kemi VE, Rita HR, Kärkkäinen MUM, Viljakainen HT, Laaksonen MM, Outila TA, et al.
455 Habitual high phosphorus intakes and foods with phosphate additives negatively affect serum
456 parathyroid hormone concentrations: a cross-sectional study on healthy premenopausal women.
457 Public Health Nutr 2009;12:1885-92.
458 [28] Tucker KL, Morita K, Qiao N, Hannan MT, Cupples LA, Kiel DP. Colas, but not other
459 carbonated beverages, are associated with low bone mineral density in older women: The
460 Framingham Osteoporosis Study. Am J Clin Nutr 2006;84:936-42.
461 [29] Itkonen ST, Karp HJ, Kemi VE, Kokkonen EM, Saarnio EM, Pekkinen MH, et al.
462 Associations among total and food additive phosphorus intake and carotid intima-media
463 thickness – a cross-sectional study in a middle-aged population in Southern Finland. Nutr J
464 2013;12:94.
465 [30] Poggio ED, Nef PC, Wang X, Greene T, Van Lente F, Dennis VW, et al. Performance of
466 the Cockroft-Gault and modification of diet in renal disease equations in estimating GRF in ill
467 hospitalized patients. Am J Kidney Dis 2005;46:242-52.

[31] Laaksonen MML, Sievänen H, Tolonen S, Mikkilä V, Räsänen L, Viikari J, et al. Determinants of bone strength and fracture incidence in adult Finns: Cardiovascular Risk in Young Finns Study (the GENDI pQCT study). Arch Osteoporos 2010;5:119-30.

[32] Sievänen H, Koskue V, Rauhio A, Kannus P, Heinonen A, Vuori I. Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. J Bone Miner Res 1998;13:871-82.

[33] Burtis CA, Ashwood ER, editors. Tietz Fundamentals of Clinical Chemistry, 5th edition. Philadelphia; WB Saunders Company: 2001, p. 802-3.

[34] Blass KG, Thibert RJ, Lam LK. Study of mechanism of Jaffe reaction. Z Klin Chem Klin Biochem 1974;12:336-343.

[35] Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: Current procedures, performance characteristics and limitations. Steroids 2010;75:477-88.

[36] Kao PC, van Heerden JA, Grant CS, Klee GG, Khosla S. Clinical performance of parathyroid hormone immunometric assays. Mayo Clin Proc 1992;67:637-45.

[37] Koivula M-K, Richardson J, Leino A, Valleala H, Griffiths K, Barnes A, et al. Validation of an automated intact N-terminal propeptide type I procollagen (PINP) assay. Clin Biochem 2010;43:1453-7.

[38] Michelsen J, Wallaschofski H, Friedrich N, Spielhageb C, Rettig R, Ittermann T, et al. Reference intervals for serum concentrations of three bone turnover markers for men and women. Bone 2013;57:399-404.

[39] Babbie ER. Analysis of data: Quantitative and qualitative: The elaboration model. In The Practice of Social Research, 12th edition. Belmont, CA, USA; Wadsworth Cengage Learning Inc: 2010, p.448-65.

[40] Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women: interaction of mechanical, hormonal and dietary factors. Sports Med 2005;35:779-830.

[41] IOM (Institute of Medicine Committee). Dietary Reference Intakes for Adequacy: Calcium and Vitamin D. In Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. DRI Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC; The National Academies Press: 2011, p.345-402.

[42] Tucker KL. Osteoporosis prevention and nutrition. Curr Osteoporos Rep 2009;4:111-7.

[43] Calvo MS, Eastell R, Offord KP, Berstrahl EJ, Burritt MF. Circadian variation in ionized calcium and intact parathyroid hormone: evidence for sex differences in calcium homeostasis. J Clin Endocrin Metab 1991;72:69-76

[44] Sievänen H. A physical model for dual-energy X-ray absorptiometry-derived bone mineral density. Invest Radiol 2000;35:325-30.

[45] Rivadeneira F, Mäkitie O. Osteoporosis and bone mass disorders: from gene pathways to treatments. Trends Endocrinol Metab 2016;27:262-8.

Table 1. Inclusion order of the explanatory variables of bone traits and turnover markers to the models.

Starting model	Variables added to previous model			
Model 1	Model 2	Model 3	Model 4	Model 5
Phosphorus intake	**Weight** **Height**	**Age**	**Calcium intake**	**Serum 25-hydroxyvitamin D** **Physical activity** **Smoking** **Contraceptive use***

*not in the models for men

Table 2. Background, dietary and biomarker characteristics of the study subjects.

	Females (n=333)			*Males (n=179)*		
Age (years)	41.9	±	2.6	42.1	±	3
Height (m) *	1.65	±	0.06	1.79	±	0.06
Weight (kg) *	72.7	±	14.3	87.4	±	13.5
Body mass index (kg/m^2) *	26.4	±	5.2	27.2	±	4
Physical activity (min/week) * [a]	514	±	393	390	±	322
Current or former smokers (%)	46		-	56		-
Contraceptive use (%)	34		-	-		-
Energy intake (kJ/d) *	7984	±	1775	9147	±	1974
Phosphorus intake (mg/d) *	1538	±	383	1812	±	466
Calcium intake (mg/d)[b]	1202	±	430	1217	±	482
S-iPINP (ng/mL) * [c]	35.4	±	13.2	40.3	±	13.3
S-CTX (ng/mL) * [c]	0.34	±	0.15	0.48	±	0.18
Serum parathyroid hormone (ng/mL) *	57.4	±	25.3	50.8	±	22.9
Serum 25-hydroxyvitamin D (nmol/L)	55.9	±	19.8	53.1	±	18.2
Serum phosphate (mmol/L)	1.13	±	0.15	1.12	±	0.17
Estimated glomerulus filtration rate (mL/min)*	85.5	±	18.9	103.9	±	18.9

Values are means ± SD

S-iPINP = serum intact pro-collagen type I amino-terminal propeptide

S-CTX = serum collagen type 1 cross-linked C-terminal telopeptide

* p values <0.05 for difference between females and males in the Mann-Whitney U-test

[a] leisure activity or regular exercise

[b] from food and supplements

[c] n=332

Table 3. Beta coefficients for phosphorus intake in regression models for bone turnover markers and bone traits among females and males. Models are described in Table 1.

	Females (n=333)[1]					*Males (n=179)*				
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 1	Model 2	Model 3	Model 4	Model 5
Bone turnover markers										
S-CTX	-0.151 *	-0.160 *	-0.155 *	-0.111	-0.119	0.001	0.005	-0.007	-0.007	**-0.010**
S-iPINP	-0.202 **	-0.224 **	-0.224 **	-0.284 *	-0.272 *	0.117	0.124	0.105	0.115	**0.107**
Bone traits										
Bone mineral content										
Distal radius	0.168 *	0.101 *	0.102 *	0.082	0.080	0.056	0.030	**0.023**	0.039	0.031
Radial shaft	0.170 *	0.090	0.090	0.108	0.126	0.067	0.045	**0.042**	0.041	0.042
Distal tibia	0.232 **	0.154 *	0.157 *	0.097	0.074	0.115 *	0.081 *	**0.077** *	0.046	0.045
Tibial shaft	0.215 **	0.121 *	0.122 *	0.077	0.065	0.122 **	0.096 *	0.094 *	0.116 *	**0.119***
Total bone area										
Distal radius	0.129 *	0.054	0.054	0.026	0.018	0.090	**0.064**	0.063	0.146	0.145
Radial shaft	0.172 *	0.094	0.095	0.128	0.141	0.086 *	**0.059**	0.059	0.048	0.049
Distal tibia	0.177 *	0.094 *	0.094	0.049	0.031	0.157	**0.138**	0.122	0.061	0.053
Tibial shaft	0.156 *	0.055	0.056	0.005	-0.013	0.118 *	**0.090** *	0.088 *	0.108	0.112
Cortical bone area										
Distal radius	0.083	0.036	0.037	0.013	0.009	0.021	-0.001	**-0.009**	0.018	0.008
Radial shaft	0.184 *	0.102 *	0.103 *	0.104	0.114	0.074	**0.053**	0.050	0.049	0.052
Distal tibia	0.125 *	0.081	0.083	0.028	0.017	0.157 *	0.138 *	0.122 *	0.061	**0.053**
Tibial shaft	0.206 **	0.108 *	0.109 *	0.048	0.033	0.118 *	**0.090** *	0.088 *	0.108	0.112*
Bone mineral density										
Trabecular, distal radius	0.076	0.079	0.080	0.070	0.074	0.033	0.034	0.027	-0.024	**-0.032**
Cortical, radial shaft	-0.087	-0.063	-0.066	-0.012	0.017	-0.007	**-0.008**	-0.008	-0.009	-0.010
Trabecular, distal tibia	0.101	0.094	0.098	0.084	0.071	0.039	0.030	0.024	-0.002	**-0.008**
Cortical, tibial shaft	0.010	0.045	0.044	0.128	0.143	0.004	**0.006**	0.006	0.008	0.007

Values are beta coefficients for phosphorus intake

[1] for bone traits n=333, for bone turnover markers n=332
CTX carboxy-terminal collagen crosslinks, iPINP intact pro-collagen type I amino-terminal propeptide
*p<0.05, **p<0.001 for phosphorus intake in the model, biggest adjusted R^2s among models in **boldface**
all continuous variables in the models log-transformed to improve normality, contraceptive use not used in the models for men

Table 4. Adjusted coefficients of determination (R^2) or regression models, and significant *p* values in bone variables among females and males. Models are described in Table 1.

	Females (n=333)[1]					*Males (n=179)*				
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 1	Model 2	Model 3	Model 4	Model 5
Bone turnover markers										
S-CTX	0.020*	0.083*	**0.091***	0.089	0.082	0	0.037	0.088	0.083	**0.096**
S-iPINP	0.038**	**0.089****	0.087**	0.086*	0.088*	0.003	0.021	0.036	0.031	**0.033**
Bone traits										
Bone mineral content										
Distal radius	0.025*	**0.168***	0.166*	0.164	0.166	0.004	0.137	**0.144**	0.14	0.118
Radial shaft	0.026*	**0.231**	0.229	0.227	0.228	0.015	0.155	**0.157**	0.152	0.123
Distal tibia	0.051*	0.258*	0.262*	0.262	**0.279**	0.035*	0.221*	**0.229***	0.226	0.222
Tibial shaft	0.043*	**0.328***	0.327*	0.326	0.327	0.065**	0.25*	0.248*	0.244*	**0.253***
Total bone area										
Distal radius	0.014*	**0.212**	0.209	0.207	0.205	0.015	**0.132**	0.127	0.13	0.116
Radial shaft	0.027*	**0.223**	0.222	0.220	0.214	0.019*	**0.172**	0.167	0.163	0.135
Distal tibia	0.028*	**0.278***	0.276	0.275	0.271	0.011	**0.234**	0.231	0.226	0.205
Tibial shaft	0.021*	**0.348**	0.346	0.346	0.345	0.052*	**0.326***	0.322*	0.319	0.322
Cortical bone area										
Distal radius	0.004	**0.075**	0.073	0.070	0.063	0	0.054	**0.062**	0.057	0.059

Radial shaft	0.031*	**0.240***	0.238*	0.235	0.234	0.016	**0.158**	0.157	0.152	0.128
Distal tibia	0.013*	0.141	0.141	0.14	**0.163**	0.029*	0.102*	0.127*	0.124	**0.166**
Tibial shaft	0.040**	0.355*	0.354*	0.354	**0.356**	0.055*	**0.277***	0.275*	0.272	0.273*
Bone mineral density										
Trabecular, distal radius	0.003	0.047	0.045	0.042	**0.051**	0	0	0.018	0.018	**0.019**
Cortical, radial shaft	0.005	0.057	0.057	0.056	**0.069**	0	**0.067**	0.064	0.059	0.064
Trabecular, distal tibia	0.007	0.056	**0.062**	0.059	**0.062**	0.001	0.015	0.024	0.020	**0.035**
Cortical, tibial shaft	-0.003	**0.051**	0.048	0.049	0.047	0	**0.094**	0.089	0.084	0.070

Values are adjusted coefficients of determination (R^2) for each model

[1] for bone traits n=333, for bone turnover markers n=332

CTX carboxy-terminal collagen crosslinks, iPINP intact pro-collagen type I amino-terminal propeptide

*p<0.05, **p<0.001 for phosphorus intake in the model, biggest adjusted R^2's among models in **boldface**
all continous variables in the models log-transformed to improve normality, contraceptive use not used in the models for males

Table 5. Bone trait characteristics of the study subjects.

	Females (n=333)	*Males (n=179)*
Bone mineral content (mg)		
Distal radius	193.6 ± 27.0 (116.4 - 281.0)	290.4 ± 44.8 (192.0 - 444.0)
Radial shaft	189.0 ± 21.2 (125.4 - 262.2)	260.6 ± 32.3 (182.3 - 415.0)
Distal tibia	517.1 ± 68.4 (313.1 - 738.0)	694.3 ± 102.4 (459.9 - 971.8)
Tibial shaft	623.9 ± 72.7 (418.5 - 852.2)	785.6 ± 93.2 (560.0 - 1033.1)
Total bone area (mm^2)		
Distal radius	315.5 ± 47.6 (183.3 - 496.0)	418.2 ± 66.9 (261.0 - 600.0)
Radial shaft	96.3 ± 14.1 (60.0 - 149.0)	137.0 ± 19.8 (99.0 - 215.0)
Distal tibia	807.2 ± 111.3 (502.5 - 1274.0)	981.3 ± 127.3 (683.0 - 1359.5)
Tibial shaft	355.6 ± 46.7 (254.5 - 531.3)	445.5 ± 51.6 (325.3 - 580.0)
Cortical bone area (mm^2)		
Distal radius	77.6 ± 11.6 (50.8 - 122.3)	112.7 ± 20.5 (74.8 - 187.3)
Radial shaft	83.1 ± 10.0 (55.0 - 117.8)	116.8 ± 15.7 (80.5 - 188.0)
Distal tibia	172.4 ± 29.8 (87.0 - 269.8)	248.3 ± 55.2 (115.8 - 427.5)
Tibial shaft	282.6 ± 34.2 (187.8 - 418.8)	359.6 ± 44.7 (257.8 - 489.3)
Bone mineral density (mg/cm^3)		
Trabecular, distal radius	196.8 ± 28.5 (128.6 - 281.0)	227.5 ± 25.8 (163.0 - 285.0)
Cortical, radial shaft	1138.1 ± 38.2 (998.6 - 1252.4)	1117.5 ± 36.4 (999.3 - 1210.8)
Trabecular, distal tibia	215.9 ± 27.2 (129.3 - 290.6)	235.8 ± 27.4 (159.9 - 302.1)
Cortical, tibial shaft	1104.7 ± 27.4 (992.2 - 1162.2)	1093.3 ± 28.3 (1002.2 - 1151.0)

Values are means ± SD (ranges)