

Low calcium:phosphorus ratio in habitual diets affects serum parathyroid hormone concentration and calcium metabolism in healthy women with adequate calcium intake

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Excessive dietary P intake alone can be deleterious to bone through increased parathyroid hormone (PTH) secretion, but adverse effects on bone increase when dietary Ca intake is low. In many countries, P intake is abundant, whereas Ca intake fails to meet recommendations; an optimal dietary Ca:P ratio is therefore difficult to achieve. Our objective was to investigate how habitual dietary Ca:P ratio affects serum PTH (S-PTH) concentration and other Ca metabolism markers in a population with generally adequate Ca intake. In this cross-sectional analysis of 147 healthy women aged 31–43 years, fasting blood samples and three separate 24-h urinary samples were collected. Participants kept a 4-d food record and were divided into quartiles according to their dietary Ca:P ratios. The 1st quartile with Ca:P molar ratio ≤ 0.50 differed significantly from the 2nd (Ca:P molar ratio 0.51–0.57), 3rd (Ca:P molar ratio 0.58–0.64) and 4th (Ca:P molar ratio ≥ 0.65) quartiles by interfering with Ca metabolism. In the 1st quartile, mean S-PTH concentration ($P=0.021$) and mean urinary Ca (U-Ca) excretion were higher ($P=0.051$) than in all other quartiles. These findings suggest that in habitual diets low Ca:P ratios may interfere with homeostasis of Ca metabolism and increase bone resorption, as indicated by higher S-PTH and U-Ca levels. Because low habitual dietary Ca:P ratios are common in Western diets, more attention should be focused on decreasing excessively high dietary P intake and increasing Ca intake to the recommended level.

Ca:P ratio: Parathyroid hormone: Ca metabolism: P intake: Ca intake

Osteoporosis is a major public health problem and a costly disease worldwide. From a nutritional point of view, the importance of adequate vitamin D and Ca intake for bone health is well established^(1–3). Nevertheless, less consensus exists about whether the intake of Ca and P, the main bone-forming minerals, must exist in a certain ratio in adult diets to ensure optimal bone health. Based on calculations of recommended dietary Ca and P intakes, the optimal dietary Ca:P molar ratio is suggested to be 1^(4–7), corresponding to a Ca:P weight ratio of 1:3. However, in many countries, dietary P intake is two- to threefold higher than recommended levels^(8–11), while total dietary Ca intake remains below nutritional recommendations^(11–16). These dietary habits lead to the lower dietary Ca:P ratios observed in various countries in recent years^(8,11,17–19).

The hypothesis of adverse effects of diets with a low Ca:P ratio originated from animal model studies, where animals fed a low Ca:P ratio diet manifested secondary hyperparathyroidism, loss of bone and osteopenia⁽⁶⁾. In addition,

a high Ca:P ratio due to low P intake was found to be favourable for bone mineralisation in adult rats, as it increased Ca absorption⁽²⁰⁾. Relative to the number and quality of animal studies, the effects of dietary Ca:P ratios on Ca and bone metabolism in human subjects have been investigated little. Furthermore, whether the dietary Ca:P ratio is clinically significant in adult human subjects has been debated^(21,22). Some earlier findings in young females have supported the importance of a sufficient dietary Ca:P ratio in bone health in epidemiological^(23,24), intervention⁽²⁵⁾ or short-term controlled studies^(26,27).

Parathyroid hormone (PTH) is a major regulator of Ca, P and bone metabolism. Ca and P have opposite effects on serum PTH (S-PTH) concentration, as dietary P has been found to increase S-PTH concentrations^(25,26) by decreasing serum-ionised Ca (S-iCa) concentration⁽²⁸⁾ and by directly affecting PTH secretion⁽²⁹⁾, probably through the Na-phosphate co-transporter in the parathyroid gland⁽³⁰⁾, while Ca administration has been demonstrated to decrease S-PTH^(31,32) through

Abbreviations: ANCOVA, analysis of covariance; LSD, least significant difference method; PTH, parathyroid hormone; S-Ca, serum Ca; S-iCa, serum-ionised Ca; S-Pi, serum phosphate; S-PTH, serum PTH; S-25(OH)D, serum 25-hydroxy vitamin D; U-Ca, urinary Ca; U-Pi, urinary phosphate.

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an increase in S-iCa⁽²⁸⁾. Intermittent administration of PTH stimulates bone formation⁽³³⁾ and increases trabecular bone mass⁽³⁴⁾, while continuous excessive PTH secretion increases bone turnover^(35,36) and releases Ca and P from bone.

Ca and P metabolism is tightly bound together, which is reflected in, for instance, the S-PTH-mediated regulation of urinary Ca (U-Ca) and urinary phosphate (U-Pi) excretions. In normal physiological conditions, an increase in S-PTH concentration results in lower U-Ca and higher U-Pi excretion. Understandably, as both dietary Ca and P intakes affect S-PTH, an increase in dietary Ca intake elevates U-Ca excretion^(27,37), while high P intake *per se*^(5,38) or together with low Ca intake^(26,27) decreases U-Ca excretion. Decreased S-PTH concentration, in turn, leads to higher U-Ca and lower U-Pi excretion. U-Pi excretion diminishes, as decreased S-PTH inhibits the activity of Na-phosphate co-transporters in kidneys⁽³⁹⁾.

Our objective here was to examine how habitual dietary Ca:P ratio affects S-PTH concentration and other Ca metabolism markers in healthy premenopausal women. We hypothesised that low dietary Ca:P ratios have a more deleterious impact than high Ca:P ratios on Ca metabolism. Since dietary Ca intake in Scandinavian countries generally meets nutritional recommendations, we were able to determine whether the optimal dietary Ca:P molar ratio of 1 is achieved among participants whose habitual dietary Ca intake is at the recommended level (≥ 800 mg/d)⁽⁴⁾.

Participants and methods

Participants

Our participants represent a subgroup of randomly selected 31- to 43-year-old Finnish women. Details about these cross-sectional study participants have been provided elsewhere⁽⁴⁰⁾. Only women with no illnesses or medications affecting Ca metabolism were included. Women with no or irregular menstruation as well as those with incomplete 4-d food records were excluded. Our final study group comprised 147 healthy premenopausal women, whose oestrogen status was presumed to be reasonable normal due to regular menstruation. Before the study, each participant gave her informed consent to the procedures, which were conducted in accordance with the Helsinki Declaration. The Helsinki University Ethics Committee approved the study protocol.

Questionnaire and dietary assessment

A questionnaire was used to collect information on weight, height, smoking habits and alcohol consumption of participants. In addition, the questionnaire requested information on age, age at menarche, past medical history (e.g. contraceptives used) and menstruation cycle.

To gather data on habitual energy and nutrient intakes, participants were instructed on how to keep a 4-d food record, which included three weekdays and 1 d of the weekend. Participants were advised to maintain their habitual food intakes during this period and to record all foods and beverages immediately after consumption. A nutritionist together with the participant checked the 4-d food record. We calculated participants' habitual dietary intakes with a

computer-based program, the Unilever Dietary Analysis Program (Becel Palvelu Paasivaara Oy, Finland, 1989), based on the food composition database (Fineli) of the Finnish National Public Health Institute.

Study design

Participants' dietary Ca:P ratios were calculated based on the information of Ca (mg/d) and P (mg/d) intakes provided in the 4-d food record. Weight Ca:P ratios were converted into molar ratios by using molecular weight of Ca (40.08 g/mol) and P (30.97 g/mol). Both molar and weight ratios of each Ca:P quartile are presented in Table 1. To examine the effects of different dietary Ca:P ratios on S-PTH and Ca metabolism markers, we divided participants into quartiles according to their dietary Ca:P molar ratios. Characteristics of quartiles are presented in Table 1. In addition, since we found that the 1st quartile differed from all other quartiles in effects on S-PTH and several other Ca metabolism markers and the 2nd, 3rd, and 4th quartiles had similar effects, we also compared the 1st quartile to the combined group of the other quartiles.

Sampling and biochemical measurements

On the study days, blood samples were taken anaerobically between 07.30 hours and 09.15 hours after a 12-h overnight fast. In addition, 24-h urine was collected three times during the study. The urine and separated serum samples were stored at -20°C until analysed. The S-iCa concentration was analysed with an ion-selective analyser (Microlyte 6, Thermo Electron Corporation, Vantaa, Finland). The intra-assay CV was 1.6% for S-iCa. The serum-intact PTH concentration was measured using an immunoradiometric assay (Nichols Institute, Juan San Capistrano, CA, USA), with 10–65 ng/l as the reference range. Intra- and interassay CV for serum-intact PTH were 3.7 and 1%, respectively. The serum 25-hydroxy vitamin D (S-25(OH)D) concentration was measured by a RIA (Dia Sorin, Inc., Stillwater, MN, USA). The intra- and interassay CV were 10.1 and 14.9%, respectively. Serum Ca (S-Ca) and phosphate (S-Pi), and U-Ca and phosphate (U-Pi) concentrations were measured by routine laboratory methods using an Elan Automatic Analyser (Eppendorf–Netheler–Hinz GmbH, Hamburg, Germany). CV % for S-Ca and U-Ca was 1.20–1.60%, and for S-Pi and U-Pi it was 2.90%. To calculate U-Ca and U-Pi values, the averages of three separate urine collections, corrected with urinary creatinine excretion, were used.

Statistical analysis

Statistical approaches. Power calculation based on S-PTH concentration (expected difference between quartiles in mean S-PTH concentration 12 ng/l), assuming 90% power with $\alpha = 0.05$, indicated that a sample size of eleven in each group was adequate⁽⁴¹⁾. Subject characteristics between quartiles were compared by ANOVA. The differences in the averages of the dependent variables between the Ca:P quartiles were compared with ANOVA and analysis of covariance (ANCOVA). ANCOVA was used because it enables inclusion of both categorical (e.g. use of contraceptives) and continuous

Table 1. Basic characteristics of all study participants (*n* 147) and of participants in each dietary molar Ca:P quartile (Mean values of daily nutrient intakes with their standard errors)

Variable	All (<i>n</i> 147)		1st Quartile* (<i>n</i> 38)		2nd Quartile† (<i>n</i> 36)		3rd Quartile‡ (<i>n</i> 39)		4th Quartile§ (<i>n</i> 34)		P (ANOVA)
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Age (years)	37.7	0.27	37.4	0.51	37.6	0.53	38.5	0.54	37.3	0.58	0.37 (NS)
BMI (kg/m ²)	23.3	0.28	23.3	0.61	23.1	0.50	23.5	0.51	23.3	0.62	0.97 (NS)
Contraceptive users (%)	26.5		26.3		19.4		28.2		32.4		0.67 (NS)
Dietary energy intake (MJ/d)	7.9	0.19	7.7	0.42	7.5	0.32	8.5	0.42	8.0	0.33	0.34 (NS)
Dietary protein intake (g/d)	73.3	1.60	73.9	3.5	66.2	2.6	77.9	2.7	74.6	3.7	0.062 (NS)
Dietary Na intake (g/d)	1.62	0.09	1.87	0.18	1.37	0.15	1.46	0.16	1.82	0.19	0.084 (NS)
Dietary Ca intake (mg/d)	1056	33.6	742	41.0	908	40.5	1253	45.3	1339	84.6	< 0.0001
Dietary Ca intake < 800 mg/d (% of participants)	29.9		68.4		36.1		0		14.7		< 0.0001
Dietary P intake (mg/d)	1411	32.7	1319	61.9	1299	56.5	1582	54.2	1438	80.7	0.005
Dietary P intake from milk and cheese (mg/d)	414.8	19.9	312.1	51.2	358.4	29.9	508.5	28.1	483.4	40.7	0.0005
Dietary Ca:P (mg:mg)	0.74	0.01	0.56	0.014	0.70	0.004	0.79	0.004	0.92	0.013	< 0.0001
Serum 25-OH vitamin D (nmol/l)	47.7	3.0	44.0	5.0	47.0	5.4	43.2	4.5	57.6	8.6	0.31 (NS)

NS (*P* > 0.05) with ANOVA.

* Ca:P molar ratio ≤ 0.50 (corresponds to Ca:P weight ratio ≤ 0.65).

† Ca:P molar ratio 0.51–0.57 (corresponds to Ca:P weight ratio 0.66–0.74).

‡ Ca:P molar ratios 0.58–0.64 (corresponds to Ca:P weight ratio 0.75–0.83).

§ Ca:P molar ratio ≥ 0.65 (corresponds to Ca:P weight ratio ≥ 0.84).

|| Between the Ca:P quartiles with ANOVA.

(e.g. S-iCa) explanatory variables. Furthermore, ANCOVA was used to exclude the possibility that differences found with ANOVA are not due to differences in relevant covariates. For pairwise comparison when ANOVA or ANCOVA *P*-values were below 0.05, Fisher's least significant difference method (Fisher's LSD) was used. We used an SPSS software program (version 15.0, SPSS, Inc., Chicago, IL, USA, 2006) in a Windows environment for all statistical analyses. Results were considered statistically significant at *P* < 0.05. Data are presented as means and standard error of means.

Covariates. Several factors are known to affect S-PTH concentration. Among these are serum variables (S-25(OH)D and S-iCa) and the use of contraceptives⁽⁴²⁾. When the means of S-iCa, S-Ca and S-Pi were compared, S-PTH was included in covariates (Table 2). In addition, S-Pi was included in covariates when comparing the means of S-iCa and S-Ca (Table 2). Na intake is known to affect U-Ca and U-Pi excretion; therefore, when the means of U-Pi and U-Ca were compared, Na intake was included in covariates. We excluded age from the covariates, as all participants represented the same age group and all were premenopausal females. Energy intake was also excluded because Ca and P intakes correlated well with energy intake. Since body weight and BMI had no effects on the variables measured, they were excluded from the covariates. In summary, we chose covariates that correlated with the outcome parameter or covariates known to affect outcome according to the literature.

Results

Baseline characteristics

The baseline characteristics of the 147 participants are displayed in Table 1.

Habitual dietary calcium and phosphorus intake. Participants' average dietary intake of Ca and P, and P intake from milk and cheese are presented in Table 1. Forty-four participants had a dietary Ca intake below recommended levels^(4,43); their mean intake was 647 mg/d (SEM 21.6). In the Ca:P quartiles, the number of participants with a dietary Ca intake below recommendations varied, being the highest in the 1st quartile (*n* 26, *n* 13, *n* 0 and *n* 5 in the 1st, 2nd, 3rd and 4th quartiles, respectively; Table 1). Among all study participants, the lowest individual dietary Ca intake was 200 mg/d and the highest was 2739 mg/d, while the lowest individual P intake corresponded exactly to nutritional recommendations for P^(4,43) and the highest (3010 mg/d) exceeded the recommendations fivefold⁽⁴³⁾. The 5th, 50th and 95th percentiles of Ca and P intake in each Ca:P quartile are presented in Fig. 1.

Habitual dietary calcium:phosphorus ratio. In line with the National FINDIET 2007 Survey results⁽⁸⁾, the average habitual dietary Ca:P ratio of our participants (Table 1) corresponded to the ratio in Finnish females. The molar ratio of dietary Ca:P varied between 0.22 and 0.91, corresponding to a Ca:P weight ratio of 0.29–1.18; thus, none of the participants achieved the suggested dietary Ca:P molar ratio of 1^(4–7). The mean molar Ca:P ratios of study participants in each quartile of dietary Ca and P intakes are presented in Fig. 2. Among all participants, the Ca:P molar ratio at the

Table 2. Mean values of serum-ionised calcium (S-iCa), calcium (S-Ca) and phosphate (S-Pi) concentrations (Mean values with their standard errors)

Quartiles and mean	S-iCa (mmol/l)		S-Ca (mmol/l)		S-Pi (mmol/l)		Number of participants
	Mean	SEM	Mean	SEM	Mean	SEM	
1st Quartile	1.204*	0.005	2.445†	0.025 NS	1.284‡	0.03 NS	38
2nd Quartile	1.195*	0.005	2.378†	0.025 NS	1.294‡	0.03 NS	36
3rd Quartile	1.197*	0.004	2.363†	0.024 NS	1.309‡	0.03 NS	39
4th Quartile	1.212*	0.005	2.427†	0.026 NS	1.278‡	0.03 NS	34
Mean	1.202	0.005	2.403	0.025	1.293	0.03	147
Reference range§	1.18–1.30		2.15–2.51		0.85–1.50		

ANCOVA, analysis of covariance; S-PTH, serum parathyroid hormone; S-25(OH)D, serum 25-hydroxy vitamin D; S-Pi, serum phosphate.

* $P < 0.05$ with ANCOVA adjusted for S-PTH, S-25(OH)D and S-Pi concentrations, and use of contraceptives.

† NS ($P > 0.05$) with ANCOVA adjusted for S-PTH, S-25(OH)D and S-Pi concentrations, and use of contraceptives.

‡ NS ($P > 0.05$) with ANCOVA adjusted for S-PTH, S-iCa and S-25(OH)D concentrations, and use of contraceptives.

§ Medix Laboratoriot (<http://www.medix.fi>).

5th percentile intake was 0.38 (weight ratio 0.49) and at the 95th percentile intake was 0.74 (weight ratio 0.96).

Effect of dietary calcium:phosphorus ratios on serum parathyroid hormone. The mean S-PTH concentration differed significantly between the Ca:P quartiles ($P = 0.014$, ANOVA), being higher in the 1st quartile than in the others. To exclude the possibility that these differences are not due to differences in relevant covariates, we adjusted the S-PTH means for the following variables (S-iCa and S-25(OH)D concentrations and use of contraceptives). After these adjustments, the S-PTH differences remained practically the same, as did their statistical significance ($P = 0.021$, ANCOVA).

The mean S-PTH values and the statistical differences of the 2nd, 3rd and 4th quartiles from the 1st quartile are shown in Fig. 3. The mean S-PTH concentration was 30% higher in the 1st quartile than in the combined group of the 2nd, 3rd and 4th quartiles ($P = 0.002$). No differences in the mean S-PTH concentrations were present among the 2nd, 3rd and 4th quartiles, and the smallest P -value among quartiles in the ANCOVA model was only 0.88.

Effect of dietary calcium:phosphorus ratios on urinary excretion of calcium and phosphate. The dietary Ca:P ratio significantly affected the 24-h U-Ca excretion ($P = 0.047$, ANOVA). The mean U-Ca excretion in the 1st quartile was

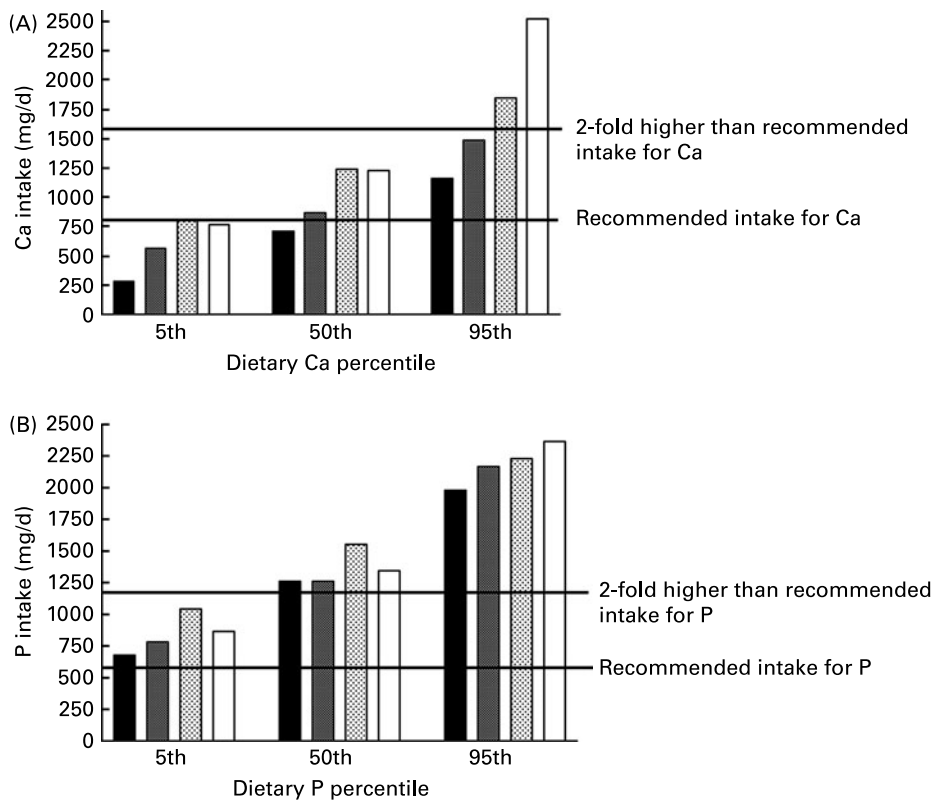


Fig. 1. The 5th, 50th and 95th percentiles of dietary calcium (mg/d; panel A) and phosphorus (mg/d; panel B) intakes in each calcium:phosphorus quartile. Calcium:phosphorus molar ratios were ≤ 0.50 , 0.51–0.57, 0.58–0.64 and ≥ 0.65 in the 1st, 2nd, 3rd and 4th quartiles, respectively. ■, 1st quartile; ▨, 2nd quartile; ▩, 3rd quartile; □, 4th quartile.

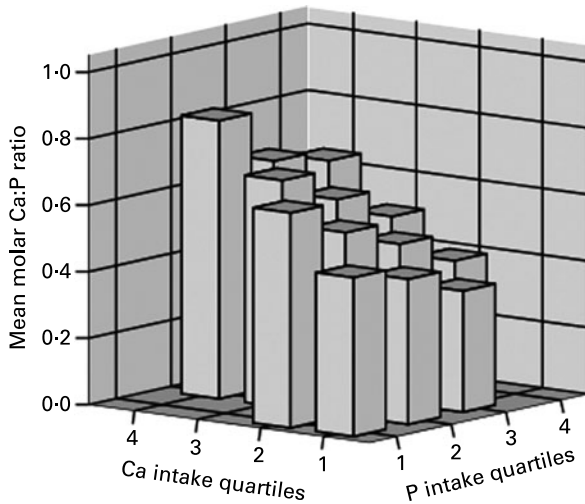


Fig. 2. The mean molar dietary calcium:phosphorus ratios (*y*-axis) in each calcium (*x*-axis) and phosphorus (*z*-axis) intake quartile. Dietary calcium intake in calcium quartiles was <770, 771–997, 998–1251 and >1251 mg/d in the 1st, 2nd, 3rd and 4th quartiles, respectively. Dietary phosphorus intake in phosphorus quartiles was <1122, 1122–1347, 1348–1643 and >1643 mg/d in the 1st, 2nd, 3rd and 4th quartiles, respectively.

higher than in the other quartiles. After adjusting the U-Ca means for the following relevant covariates (dietary Na and protein intakes, S-PTH and S-25(OH)D concentrations, and use of contraceptives), similar effects remained in the U-Ca differences and in their statistical significance ($P=0.051$, ANCOVA). The mean U-Ca values are shown in Fig. 4. When comparing the 2nd, 3rd and 4th quartiles as a combined group with the 1st quartile, the mean U-Ca excretion was about 30% higher in the latter ($P=0.006$, ANCOVA). In addition, the mean U-Ca excretion did not vary between the 2nd, 3rd and 4th quartiles (ANCOVA; $P=0.58-0.90$).

The mean 24-h U-Pi excretion did not differ between the Ca:P quartiles ($P=0.33$, ANOVA). However, after adjustment for relevant covariates (dietary Na intake, S-PTH and S-25(OH)D concentrations, and use of contraceptives), the mean U-Pi excretion tended to be different between the quartiles ($P=0.13$, ANCOVA). In the 1st quartile, U-Pi excretion was 6–17% higher than in the 2nd, 3rd and 4th quartiles. Furthermore, when comparing the 2nd, 3rd and 4th quartiles as one group with the 1st quartile, the mean U-Pi excretion was about 13% higher in the 1st quartile ($P=0.055$, ANCOVA).

Effect of dietary calcium:phosphorus ratios on serum-ionised calcium, calcium and phosphate. The mean S-iCa concentration differed significantly between the quartiles ($P=0.027$, ANOVA), being the highest in the 4th quartile. S-iCa concentration in the 4th quartile differed significantly from all other quartiles ($P=0.050$, $P=0.006$ and $P=0.013$ in the 2nd, 3rd and 4th quartiles, respectively, Fisher's LSD comparisons, ANOVA). After adjusting the S-iCa means for the relevant variables (S-PTH, S-25(OH)D and S-Pi concentrations, and use of contraceptives), the S-iCa differences remained practically the same, as did their statistical significance ($P=0.040$, ANCOVA; Table 2). However, the 1st quartile did not differ significantly from the 4th quartile ($P=0.242$, Fisher's LSD comparisons, ANCOVA), although the 2nd ($P=0.010$, Fisher's LSD comparisons, ANCOVA)

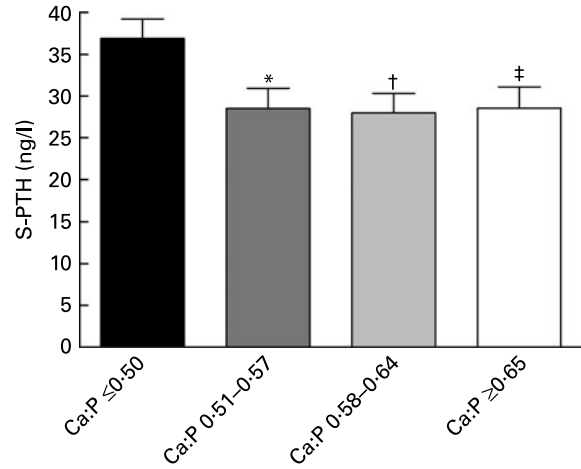


Fig. 3. Effects of calcium:phosphorus ratios on serum parathyroid hormone (S-PTH) concentration (means with their standard errors) in the different quartiles. The covariates included serum 25-hydroxyvitamin D and ionised calcium concentrations, and use of contraceptives. Analysis of covariance (ANCOVA) was performed. Mean value was significantly different from that of the 1st quartile: * $P=0.014$, † $P=0.07$, ‡ $P=0.017$ (Fisher's least significant difference comparison, ANCOVA). ■, 1st quartile; ■, 2nd quartile; □, 3rd quartile; □, 4th quartile.

and 3rd ($P=0.022$, Fisher's LSD comparisons, ANCOVA) quartiles did (Table 2).

While no differences existed in S-Ca concentration between the Ca:P quartiles by ANOVA ($P=0.27$), S-Ca concentration tended to differ between quartiles ($P=0.061$, ANCOVA) after adjustment for relevant covariates (S-PTH, S-25(OH)D and S-Pi concentrations, and use of contraceptives; Table 2).

The mean S-Pi concentration did not differ between the quartiles ($P=0.85$, ANOVA). After adjusting the S-Pi means for relevant covariates (S-PTH, S-25(OH)D and S-iCa concentrations, and use of contraceptives), no differences between the quartiles were found ($P=0.87$, ANCOVA; Table 2).

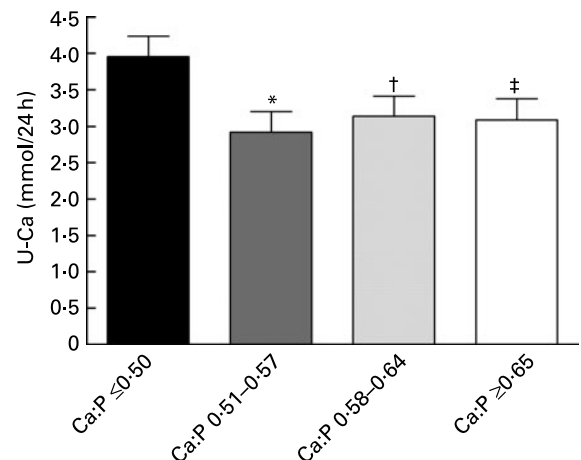


Fig. 4. Effects of calcium:phosphorus ratios on 24-h urinary calcium (U-Ca) excretion (means with their standard errors) in the different quartiles. The covariates included serum 25-hydroxyvitamin D and parathyroid hormone concentrations, dietary sodium and protein intakes, and use of contraceptives. Analysis of covariance (ANCOVA) was performed. Mean value was significantly different from that of the 1st quartile: * $P=0.010$, † $P=0.040$, ‡ $P=0.035$ (Fisher's least significant difference comparison, ANCOVA). ■, 1st quartile; ■, 2nd quartile; □, 3rd quartile; □, 4th quartile.

Discussion

In this cross-sectional study, low habitual dietary Ca:P ratios interfered with Ca metabolism in healthy women with an adequate dietary Ca intake. Interestingly, the lowest quartile (Ca:P molar ratio ≤ 0.50) differed from all other quartiles by increasing both S-PTH concentration and U-Ca excretion. In fact, quartiles other than 1st one had similar effects on the markers measured, except on S-iCa concentration. The present findings imply that a cut-off Ca:P ratio may exist, lower than suggested Ca:P molar ratio of 1⁽⁴⁻⁷⁾, below which the effects on mineral metabolism and bone health are more severe. It is noteworthy that dietary Ca intake among our participants was mostly adequate or high (mean Ca intake 1056 mg/d). Even in the 1st quartile, the mean Ca intake was nearly 750 mg/d. This intake reflects the high dairy product consumption of participants. More details of participants' consumption of dairy products have been provided elsewhere⁽⁴⁴⁾. Nevertheless, none of the participants achieved the suggested Ca:P molar ratio of 1 in their diets. This is mainly due to the excessive P content in their habitual diets, rather than low dietary Ca intake, as mean dietary P intake exceeded 2.4-fold, and mean dietary Ca intake 1.3-fold the Nordic nutritional recommendations for P (600 mg/d) and Ca (800 mg/d)⁽⁴³⁾. These intakes corresponded to the average intake of Ca and P in Finnish females in 2007⁽⁸⁾.

Speculation has arisen as to whether the dietary Ca:P ratio is clinically significant in adult human subjects⁽²¹⁾. In infants and children, a dietary Ca:P weight ratio of 1.5 is considered to be ideal for optimal growth⁽⁹⁾. However, in adult diets, the Ca:P ratio varies, being highest in dairy products⁽⁴⁵⁾. P is present in a wide range of foods, while about 75% of Ca comes from dairy products⁽⁸⁾. In addition, P is added to foods as a phosphate-containing food additive, further increasing already high P intake. Although, some foods rich in P are also good sources of Ca, e.g. dairy products, many others contain very little Ca. Among the participants in the present study, the intake of P from milk and cheese was higher in the 3rd and 4th Ca:P quartiles indicating that dairy products represent a higher proportion of the total P intake in these quartiles. Therefore, if the habitual diet lacks dairy products, the dietary Ca:P ratio will easily drop below optimal levels. Very low Ca:P ratios (≤ 0.25) have been reported in diets of young girls and boys in Poland⁽¹⁸⁾, as well as in teenagers and young adults in USA⁽¹¹⁾.

Calvo *et al.*⁽⁴⁶⁾ found that a high-P (1700 mg/d), low-Ca diet (400 mg/d) consumed by young adults for 8 d resulted in elevated S-PTH. Interestingly, S-PTH concentrations increased more in females than in males. In an additional 4-week study in young females only, S-PTH levels increased in response to a high-P, low-Ca diet, but no changes occurred in another central regulator of Ca and bone metabolism, serum 1,25-(OH)₂D concentration, which usually increases in response to low Ca intake⁽²⁵⁾. The authors suggested that the usual homeostatic mechanism used when dietary Ca is limited is disturbed by chronic high P intake. Similar results were observed in a 24-h controlled study in healthy young females, whose S-PTH concentrations increased in a dose-dependent manner in response to increasing P intake and decreasing Ca:P ratio, although no changes in serum 1,25-(OH)₂D concentrations occurred⁽²⁶⁾. Unfortunately, serum 1,25-(OH)₂D concentrations were not

measured in the present study, but in line with observations in earlier interventions^(25,46) and short-term controlled studies^(26,27), higher S-PTH concentrations were found in participants with low dietary Ca:P ratios (Ca:P molar ratio ≤ 0.50). While the mean intake of P in all quartiles was over twofold higher (> 1200 mg/d) than dietary guidelines^(4,43), the mean intake of Ca was slightly below recommended levels^(4,43) in the 1st quartile only (742 mg/d). In fact, excluding the 1st quartile revealed similar effects on S-PTH in the other quartiles. This similarity may be explained by higher intake of Ca, as Ca intake has been observed to decrease absorption of P in the intestine⁽⁴⁷⁾ and to suppress S-PTH concentration induced by high P intake⁽²⁷⁾. S-PTH concentration is apparently lower in the mornings⁽²⁸⁾. In line with this, we have previously demonstrated that P-containing meals increase S-PTH above the higher reference limit on study days, with S-PTH values remaining elevated the following mornings⁽²⁶⁾, indicating the presence of a transient secondary hyperparathyroidism. In the present study, higher S-PTH concentrations could have been noted if samples other than morning fasting samples had also been collected. Nevertheless, the finding of increased mean S-PTH only in the 1st quartile supported the importance of higher dietary Ca:P ratios and the vital role of adequate Ca intake simultaneously with high dietary P intake in habitual diets.

An unexpected physiological increase in 24-h U-Ca excretion was observed in the 1st quartile. This was unexpected, since the mean dietary Ca intake and Ca:P ratio in the 1st quartile were the lowest. Furthermore, the mean S-PTH concentration in this quartile was the highest. The present finding is interesting, as in normal physiological conditions elevated S-PTH leads to decreased U-Ca excretion. In primary hyperparathyroidism, an increase in U-Ca excretion is, however, often seen⁽⁴⁸⁾. The U-Ca finding suggests that low dietary Ca:P ratios in habitual diets somehow interfere with the homeostasis of Ca metabolism. An increase in S-PTH due to a low Ca:P ratio would be expected to increase bone resorption, and some of the extra Ca, which has been released from bone, to be excreted in urine. Elevated U-Ca excretion might therefore reflect an increase in bone resorption.

U-Ca excretion is an important determinant of Ca retention in the healthy human body. In a controlled situation, U-Ca excretion has been strongly correlated with acute Ca intake⁽³⁷⁾. In a controlled short-term study, when dietary P intake was high (1850 mg/d), U-Ca excretion increased with increasing dietary Ca intake and Ca:P ratio in a dose-dependent manner in healthy women⁽²⁷⁾. In addition, 24-h U-Ca excretion was similar with P intakes of 1245 and 1995 mg/d when Ca intake was very low (250 mg/d). These findings indicate that U-Ca excretion might not diminish after a certain high P intake, which may result in an unfavourable Ca balance⁽²⁶⁾. Moreover, low Ca intake and high U-Ca excretion have been assumed to reduce Ca accretion in bone of young adults during growth and to have a negative impact on skeletal development⁽⁴⁹⁾. In the present study, U-Ca excretion was expected to be the highest in the 3rd and 4th quartiles, as dietary P intake was high in all of the quartiles, but dietary Ca intake was significantly higher only in the 3rd and 4th quartiles. Furthermore, in the 1st quartile, the mean Ca intake was slightly below recommended levels (≥ 800 mg/d)^(4,43).

In fact, after excluding the 1st quartile, the other quartiles were highly similar in their effects on U-Ca excretion. This implies that in habitual diets with excessive dietary P intake an adequate dietary Ca intake is needed to overcome the interfering effects of low Ca:P ratios on Ca excretion.

In summary, low dietary Ca:P ratios in habitual diets of healthy women affected both S-PTH and Ca metabolism. Interestingly, the lowest Ca:P quartile differed from all other quartiles in a negative manner. The results suggested that such low Ca:P ratios (Ca:P molar ratio ≤ 0.50) in diets may disturb Ca metabolism and negatively affect bone in healthy subjects, as indicated by higher S-PTH and U-Ca levels together. However, whether it is necessary to reach a Ca:P molar ratio of 1 in diets is unknown. Even participants with a high dietary Ca intake did not achieve the suggested Ca:P ratio of 1. The present results imply that a Ca:P molar ratio higher than 0.50 is sufficient when dietary Ca intake is at the recommended level. However, higher Ca:P ratios might be needed if dietary Ca intake drops markedly below the nutritional recommendations. The present study also indicated that consumption of a large excess of dietary P is not optimal for mineral metabolism and bone health. In Western diets, low Ca:P ratios are common. Thus, more attention should be focused on decreasing excessively high P intake and increasing Ca intake to meet dietary guidelines. In Western diets, dairy products consumption will easily ensure adequate Ca intake. High dietary P intake, in turn, will be reduced by restricting the consumption of highly processed foods and increasing the consumption of raw and unprocessed foods. Nevertheless, increasing dietary Ca intake far beyond the nutritional recommendations should be avoided, as it might lead to other health risks. Identifying persons at risk of having low Ca:P ratios in their habitual diets would be useful. Also, because dietary Ca intake was generally adequate among our participants, additional studies are needed to investigate the impact of dietary Ca:P ratios in populations where low dietary Ca intake is common.

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References

1. Tang BPM, Eslick GD, Nowson C, *et al.* (2007) Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* **370**, 657–666.

2. Bischoff-Ferrari HA, Willett WC, Wong JB, *et al.* (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* **293**, 2257–2264.
3. Welten DC, Kemper HCG, Post GB, *et al.* (1995) A meta-analysis of the effects of calcium intake on bone mass in young and middle aged females and males. *J Nutr* **125**, 2802–2813.
4. Advisory Committee of Government (2005) *Finnish Nutrition Recommendations – Diet and Physical Activity in Balance*. Helsinki: Edita.
5. Whybro A, Jagger H, Barker M, *et al.* (1998) Phosphate supplementation in young men: lack of effect on calcium homeostasis and bone turnover. *Eur J Clin Nutr* **52**, 29–33.
6. Calvo MS & Park YK (1996) Changing phosphorus content of the U.S. diet: potential for adverse effects on bone. *J Nutr* **126**, 1168S–1180S.
7. SCF (Scientific Committee on Food) (1993) Reports of the Scientific Committee on Food (31st Series). *Nutrient and Energy Intakes for the European Communities*, pp. 177–189. Luxembourg: Commission of the European Community. <http://europa.eu.int/comm/food/fs/sc/scf/out89.pdf>.
8. Paturi M, Tapanainen H, Reinivuo H, *et al.* (editors) (2008) The National FINDIET 2007 Survey. *Kansanterveyslaitoksen julkaisuja B23/2008*. Helsinki, Finland: Yliopistopaino.
9. European Food Safety Authority (EFSA) (2005) Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the commission related to the tolerable upper intake level of phosphorus. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_11786207671179.htm (accessed October 2008).
10. Gronowska-Senger A & Kotanska P (2004) Phosphorus intake in Poland in 1994–2000. *Rocz Państw Zakł Hig* **55**, 39–49 (abstract).
11. Calvo MS (1993) Dietary phosphorus, calcium metabolism and bone. *J Nutr* **123**, 1627–1633.
12. Henrix P, Van Cauwenbergh R, Robberrecht HJ, *et al.* (1995) Measurement of the daily dietary calcium and magnesium intake in Belgium, using duplicate portion sampling. *Z Lebensm Unters Forsch* **201**, 213–217 (abstract).
13. Bryant RJ, Cadogan J & Weaver CM (1999) The new dietary reference intakes for calcium: implications for osteoporosis. *J Am Coll Nutr* **18**, 406S–412S.
14. Guéguen L & Pointillart A (2000) The bioavailability of dietary calcium. *J Am Coll Nutr* **19**, 119S–136S.
15. Lombardi-Boccia G, Aguzzi A, Cappelloni M, *et al.* (2003) Total-diet study: dietary intakes of macro elements and trace elements in Italy. *Br J Nutr* **90**, 1117–1121.
16. Salamoun MM, Kizirian AS, Tannous RI, *et al.* (2005) Low calcium and vitamin D intake in healthy children and adolescents and their correlates. *Eur J Clin Nutr* **59**, 177–184.
17. Brot C, Jorgensen N, Madsen OR, *et al.* (1999) Relationships between bone mineral density, serum vitamin D metabolites and calcium:phosphorus intake in healthy perimenopausal women. *J Intern Med* **245**, 509–516.
18. Chwojnowska Z, Charzewska J, Chabros E, *et al.* (2002) Contents of calcium and phosphorus in the diet of youth from Warsaw elementary schools. *Rocz Państw Zakł Hig* **53**, 157–165.
19. Takeda E, Sakamoto K, Yokota K, *et al.* (2002) Phosphorus supply per capita from food in Japan between 1960 and 1995. *J Nutr Sci Vitaminol* **48**, 102–108 (abstract).
20. Koshihara M, Katsumata S-I, Uehara M, *et al.* (2005) Effects of dietary phosphorus intake on bone mineralization and calcium absorption in adult female rats. *Biosci Biotechnol Biochem* **69**, 1025–1028.
21. Sax L (2001) The Institute of Medicine's 'Dietary reference intake' for phosphorus: a critical perspective. *J Am Coll Nutr* **20**, 271–278.

22. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine & National Research Council (1997) *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press.
23. Metz JA, Anderson JJ & Gallagher PN Jr (1993) Intakes of calcium, phosphorus, and protein, and physical-activity level are related to radial bone mass in young adult women. *Am J Clin Nutr* **58**, 537–542.
24. Teegarden D, Lyle RM, McCabe GP, *et al.* (1998) Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. *Am J Clin Nutr* **68**, 749–754.
25. Calvo MS, Kumar R & Heath H 3rd (1990) Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. *J Clin Endocrinol Metab* **70**, 1334–1340.
26. Kemi VE, Kärkkäinen MUM & Lamberg-Allardt CJE (2006) High phosphorus intakes acutely and negatively affect calcium and bone metabolism in a dose-dependent manner in healthy young females. *Br J Nutr* **96**, 545–552.
27. Kemi VE, Kärkkäinen MUM, Karp HJ, *et al.* (2008) Increased calcium intake does not completely counteract the effects of increased phosphorus intake on bone: an acute dose-response study in healthy female. *Br J Nutr* **99**, 832–839.
28. Herfarth K, Schmidt-Gayk H, Graf S, *et al.* (1992) Circadian rhythm and pulsatility of parathyroid hormone secretion in man. *Clin Endocrinol* **37**, 511–519.
29. Slatopolsky E, Finch J, Denda M, *et al.* (1996) Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion *in vitro*. *J Clin Invest* **97**, 2534–2540.
30. Miyamoto K, Tatsumi S, Segawa H, *et al.* (1999) Regulation of PiT-1, a sodium-dependent phosphate co-transporter in rat parathyroid glands. *Nephrol Dial Transplant* **14**, Suppl. 1, S73–S75.
31. Kärkkäinen MUM, Lamberg-Allardt CJE, Ahonen S, *et al.* (2001) Does it make a difference how and when you take your calcium? The acute effects of calcium on calcium and bone metabolism. *Am J Clin Nutr* **74**, 335–342.
32. Sadideen H & Swaminathan R (2004) Effect of acute oral calcium load on serum PTH and bone resorption in young healthy subjects: an overnight study. *Eur J Clin Nutr* **58**, 1661–1665.
33. Liu CC & Kalu DN (1990) Human parathyroid hormone-(1-34) prevents bone loss and augments bone formation in sexually mature ovariectomized rats. *J Bone Miner Res* **5**, 973–982.
34. Hodsman AB, Steer BM, Fraher LJ, *et al.* (1991) Bone densitometric and histomorphometric responses to sequential human parathyroid hormone (1-38) and salmon calcitonin in osteoporotic patients. *Bone Miner* **14**, 67–83.
35. Tam CS, Heersche JNM, Murray TM, *et al.* (1982) Parathyroid hormone stimulates the bone apposition rate independently of its resorptive action: differential effects of intermittent and continuous administration. *Endocrinology* **110**, 506–512.
36. Schiller PC, Dippolito G, Roos BA, *et al.* (1999) Anabolic or catabolic responses of MC3T3-E1 osteoblastic cells to parathyroid hormone depend on time and duration of treatment. *J Bone Miner Res* **14**, 1504–1512.
37. Charles P (1992) Calcium absorption and calcium bio-availability. *J Intern Med* **231**, 161–168.
38. Bell RR, Draper HH, Tzeng DYM, *et al.* (1977) Physiological responses of human adults to foods containing phosphate additives. *J Nutr* **107**, 42–50.
39. Murer H, Forster I, Hernando N, *et al.* (1999) Posttranscriptional regulation of the proximal tubule NaPi-II transporter in response to PTH and dietary Pi. *Am J Physiol* **46**, F676–F684.
40. Lamberg-Allardt CJ, Outila TA, Kärkkäinen MU, *et al.* (2001) Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? *J Bone Miner Res* **16**, 2066–2073.
41. Kärkkäinen M, Outila T & Lamberg-Allardt C (1998) Habitual dietary calcium intake affects serum parathyroid hormone concentration in postmenopausal women with a normal vitamin D status. *Scand J Nutr* **42**, 104–107.
42. Teegarden D, Legowski P, Gunther CW, *et al.* (2005) Dietary calcium intake protects women consuming oral contraceptives from spine and hip bone loss. *J Clin Endocrinol Metab* **90**, 5127–5133.
43. Nordic Council of Ministers (2004) *Nordic Nutrition Recommendations 2004. Integrating Nutrition and Physical Activity*. Århus: Scanprint A/S.
44. Kemi VE, Rita HJ, Kärkkäinen MU, *et al.* (2009) Habitual high phosphorus intakes and foods with phosphate additives negatively affect serum parathyroid hormone concentration: a cross-sectional study on healthy premenopausal women. *Public Health Nutr* **12**, 1885–1892.
45. National Public Health Institute, Nutrition Unit (2008) Fineli®- Finnish Food Composition Database. Release 6. Helsinki, Finland. <http://www.fineli.fi/> (accessed September 2008).
46. Calvo MS, Kumar R & Heath H 3rd (1988) Elevated secretion and action of serum parathyroid hormone in young adults consuming high phosphorus, low calcium diets assembled from common foods. *J Clin Endocrinol Metab* **66**, 823–829.
47. Nolan CR & Cumibi WY (2003) Calcium salts in the treatment of hyperphosphatemia in hemodialysis patients. *Curr Opin Nephrol Hypertens* **12**, 373–379.
48. American Society for Bone and Mineral Research (2008) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, DC: The Sheridan Press.
49. Matkovic V, Ilich JZ & Andon MB (1995) Urinary calcium, sodium, and bone mass of young females. *Am J Clin Nutr* **62**, 417–425.