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Increased calcium intake does not completely counteract the effects of increased phosphorus intake on bone: an acute dose–response study in healthy females

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A high dietary P intake is suggested to have negative effects on bone through increased parathyroid hormone secretion, as high serum parathyroid hormone (S-PTH) concentration increases bone resorption. In many countries the P intake is 2- to 3-fold above dietary guidelines, whereas Ca intake is too low. This combination may not be optimal for bone health. In a previous controlled study, we found that dietary P dose-dependently increased S-PTH and bone resorption and decreased bone formation. The aim of the present study was to investigate the dose–response effects of Ca intake on Ca and bone metabolism with a dietary P intake higher than recommended. Each of the twelve healthy female subjects aged 21–40 years attended three 24-h study sessions, which were randomized with regard to a Ca dose of 0 (control day), 600 or 1200 mg, and each subject served as her own control. The meals on each study day provided 1850 mg P and 480 mg Ca. S-PTH concentration decreased ($P<0{\cdot}001$) and serum ionized Ca concentration increased ($P<0{\cdot}001$) with increasing Ca doses. The bone formation marker, serum bone-specific alkaline phosphatase, did not differ significantly ($P=0{\cdot}4$). By contrast, the bone resorption marker, urinary N-terminal telopeptide of collagen type I, decreased significantly with both Ca doses ($P=0{\cdot}008$). When P intake was above current recommendations, increased Ca intake was beneficial for bone, as indicated by decreased S-PTH concentration and bone resorption. However, not even a high Ca intake could affect bone formation when P intake was excessive.

Parathyroid hormone: Calcium intake: Phosphorus intake: Calcium:phosphorus ratio: Bone metabolism

Osteoporosis is a major public health problem and a costly disease worldwide[1–3]. The number of hip fractures is predicted to double in the EU nations over the next 50 years[3]. From a nutritional point of view, both of the main bone-forming minerals, Ca and P, are important for bone health, but an overly ample P intake has been suggested to be deleterious to bone through increased parathyroid hormone (PTH) secretion[4–6], especially when dietary Ca intake is low[7]. Nevertheless, no controlled studies are available on the dual effects of high P and varying Ca intakes in human subjects. The objective of our study was thus to examine in healthy females whether the effects of P intake higher than the recommended RDA[8] on bone and Ca metabolism diminish with increasing Ca intake. This study is a sequel to our recent study in which we demonstrated that P intake has adverse dose–response effects on Ca and bone metabolism in healthy young females[9]. We performed the present study with Ca and P doses normal and achievable in Western diets.

In many countries the dietary intake of P is 2- to 3-fold higher[10–13] than the recommended RDA for P intake[8]. Furthermore, the intake of P has risen during the last decades[8], with an increasing intake from processed foods with phosphate-containing food additives; thus, the dietary intake in certain groups might be several grams per day[14], even exceeding the upper reference limit of 4 g/d[8]. In addition, the total P intake is severely underestimated because the nutrition composition tables do not include P from phosphate-containing food additives[15,16].

The importance of Ca in maintaining mineral homeostasis is well established[17,18], and epidemiological studies suggest that Ca supplements prevent osteoporosis and fractures[19]. While food fortification with Ca[20,21] and Ca supplementation[22,23] has increased, total dietary Ca intake still remains below nutritional recommendations in many countries[24–28]. However, to avoid elevated health risks, it should be remembered that the safe upper intake level for Ca is 2·5 g/d, and the lowest observed adverse effect level is 5·0 g/d[8].

Abbreviations: BALP, bone-specific alkaline phosphatase; S-BALP, serum-BALP; AC and HC, treatment with adequate- and high-Ca dietary treatments respectively; PTH, parathyroid hormone; S-PTH, serum-PTH; S-iCa, serum ionized Ca; S-Pi, serum-phosphate; U-NTx, urinary N-terminal telopeptide of collagen type I.

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The hypothesis of adverse effects of high-P and low-Ca diets, i.e. diets with a low Ca:P ratio, originated from animal studies, where animals fed with a low Ca:P ratio diet manifested secondary hyperparathyroidism, loss of bone and osteopenia[7]. These were associated with increased PTH secretion and decreased serum Ca concentration. In addition, in a recent animal study by Koshihara *et al.*[29], a high Ca:P ratio was found to be favourable for bone mineralization in adult rats. Also, in a cross-sectional study in human subjects, Basabe *et al.*[30] concluded that high Ca consumption (> 1000 mg/d) and a Ca:P ratio exceeding 0·74 are associated with better bone mineral densities in young females. These findings are in accord with earlier epidemiological studies in human subjects[31,32]. In our recent controlled short-term study in healthy females, we demonstrated that dietary P has dose-dependent effects on Ca and bone metabolism by increasing S-PTH and bone resorption and decreasing bone formation[9]. In the present 24-h study, the dietary P intake was 2·5-fold higher than the current RDA[8] but normal for a Western diet, and the same level as the highest dose in our previous study[9]. We hypothesized that by increasing Ca intake the effects of higher P intake on Ca and bone metabolism would diminish.

Subjects and methods

Study subjects

We recruited fifteen women aged 21–40 years from among the students and employees of the University of Helsinki. Exclusion criteria were illnesses and medications affecting bone and mineral metabolism. Six of the subjects used oral contraceptives. The Ethical Committee of Public Health and Epidemiology in the Hospital district of Helsinki and Uusimaa approved the study protocol. Each subject gave her informed consent to the procedures, which were in accord with the Helsinki Declaration. Twelve women completed the study and three interrupted the study for personal reasons. The basic characteristics of the subjects are presented in Table 1.

Nutritional assessment

To estimate habitual energy and Ca and P intakes, we instructed the subjects on how to keep a 4-d food record, which included three weekdays and one weekend day. The subjects were advised to maintain their usual food intakes during this period and to record all foods and beverages immediately after consumption. We calculated subjects' usual dietary intakes with a computer-based program (DIET 32, version 1.22, Aivo Oy, Turku, Finland) based on the food composition database (Fineli) of the Finnish National Public Health Institute. Basic characteristics of the subjects' typical dietary intakes are shown in Table 1.

Table 1. Basic characteristics of the study subjects (*n* 12)

Variable	Mean	Range
Age (y)	24	21–40
Weight (kg)	60	46–70
Height (cm)	165	155–173
BMI (kg/m^2)	22·2	17·6–27·5
Habitual dietary energy intake (MJ/d)	7·7	6·5–9·9
Habitual dietary Ca intake (mg/d)	883	405–1501
Habitual dietary P intake (mg/d)	1247	927–1734
Habitual dietary Ca:P ratio (mg:mg)	0·70	0·37–0·87

Study protocol

Attendance and instructions. Subjects attended three 24-h study sessions over a 1-month period, with at least one week between sessions. Before the subjects came to the research unit at 08.00 hours, they fasted overnight (12 h). We instructed subjects to refrain from alcohol consumption the day before the study day because alcohol consumption has been found to affect PTH secretion[33]. In addition, we advised subjects to maintain their normal milk and milk-product consumption a few days before the study day to keep baseline levels of Ca and bone markers as similar as possible at the beginning of each study day.

Study day meals. All of the study days' meals were identical for each subject on each study day. No additional meals or snacks were allowed, but water was provided *ad libitum.* The meals provided total energy of 8·4 MJ (2000 kcal), with a P content of 1850 mg and a Ca content of 480 mg/d. Meals were normal foods purchased from Finnish grocery stores, cooked and apportioned by the same person in the research unit on each study day. The subjects ate all meals except supper in the research unit. The distribution of Ca and P contents of the study day meals is presented in Fig. 1.The main P sources were meat products (32 %), grain products (19 %), milk products (17 %) and eggs (7 %).

Study design

We gave subjects 0 (control), 600 or 1200 mg Ca as a Ca supplement ($CaCO_3$) widely available in Finland (Kalsium, Friggs, Oy Seege Ab, Helsinki, Finland) in 450 ml diluted sugar-free lemon juice (Fun Light Lemon, Felix Abba Oy Ab, Turku, Finland) during each of the three sessions. The order of the study sessions was randomized, and each subject served as her own control. The subjects received the Ca supplement in

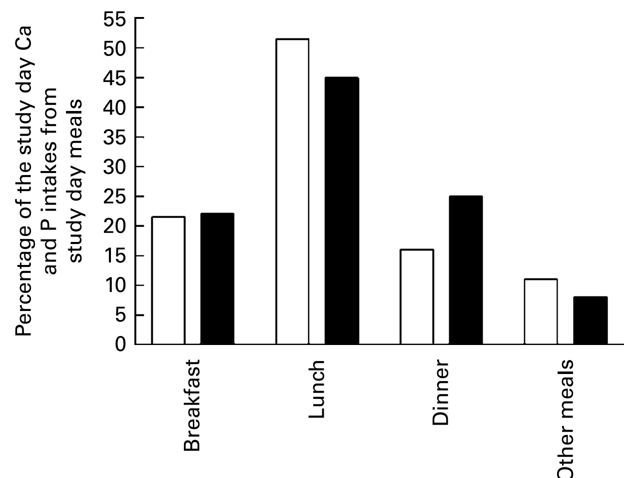

Fig. 1. Distribution of calcium (□) and phosphorus (■) content of study day meals.

three separate equally sized doses, at 08.00 hours with breakfast after the first blood sample, at 12.00 hours with lunch after the second blood sample and at 17.00 hours with dinner. Snacks were served at 14.00 hours and supper at 20.00 hours. The study design is presented in Fig. 2. On each study day, the same person apportioned the sugar-free lemon juice with and without Ca supplement. Our study design included a control day, representing low Ca (480 mg) and 2·5-fold higher P (1850 mg) intake than the current RDA[8], a 600 mg Ca day (AC), representing adequate Ca (1080 mg) and 2·5-fold higher P (1850 mg) intake than the current RDA[8], and a 1200 mg Ca day (HC), representing high Ca (1680 mg) and P (1850 mg) intake 2·5-fold above the current RDA[8]. The total Ca intakes and the dietary Ca:P ratios of subjects throughout the three study days are presented in Table 2.

Sampling

At each study session, the first blood samples were taken anaerobically at 08.00 hours after a 12-h overnight fast. Subsequent blood samples were taken at 12.00, 14.00, 16.00 and 19.00 hours. On the study day, morning urine was voided and discarded at home. Subsequent urine was collected at the research unit, with a 24-h urine collection starting at 08.00 hours, when subjects arrived at the unit, and ending at 08.00 hours the following morning. Urine and separated serum samples were stored at −20°C until analysed. The sampling procedure is presented in Fig. 2.

Laboratory methods

All samples from the same subject were analysed in the same assay in randomized order. The serum ionized Ca (S-iCa) concentration was analysed within 90 min of sample collection with an ion selective analyser (Microlyte 6, Thermo Electron Corporation, Vantaa, Finland). The S-iCa concentration has been shown to be stable within this time range[34]. The intra-assay CV was 1·6 % for S-iCa. The serum phosphate (S-Pi), total serum Ca, serum creatinine, urinary Ca, urinary phosphate, and urinary creatinine were analysed spectrometrically with an autoanalyser (Konelab 20 automatic analyser, Thermo Electron Corporation). The intra- and interassay CV for these analyses were <2·0 % and <3·5 %, respectively. The serum intact PTH concentration was determined with an Allegro intact PTH kit (Nichols Institute, San Juan Capistrano, CA, USA); the intra- and interassay CV were 1 % and 4 %, respectively. Serum bone-specific alkaline phosphatase (S-BALP) concentration was analysed from control and HC blood samples with an enzyme immunoassay (Metra™ BAP EIA Kit, Quidel Corporation, San Diego, CA, USA); the intra- and interassay CV were 5·4 % and 7·4 %, respectively. The concentration of urinary N-terminal telopeptide of collagen type ? (U-NTx) was measured with an enzyme-linked immunosorbent assay using Osteomark® NTx Test kits (Ostex International, Inc., Seattle, WA, USA); the intra- and interassay CV were 4·0 % and 6·0 %, respectively.

Statistical analysis

We used an SPSS software program (version 10.0, SPSS Inc., Chicago, IL, USA) in a Windows environment for all statistical analyses. The data are expressed as means with their standard errors. We tested the normality of variables and used logarithmic transformations when necessary to normalize non-normal distributions. ANOVA with repeated measures was used to compare study periods. The effects of Ca doses were compared with the control session using contrast analysis. We regarded a P value $\leq$ 0·05 as significant.

Results

Baseline characteristics

The baseline characteristics of study subjects are presented in Table 1.

Nutrient intakes

The average dietary intake of Ca and P of study subjects (Table 1) corresponds to the average intake of Ca and P in Finnish females[12]. Furthermore, the average dietary Ca:P ratio for study subjects was the same as the average ratio for Finnish females[12].

Markers of calcium and bone metabolism

A significant dose–response relationship was observed between S-iCa concentration and Ca intake ($P<0·001$, ANOVA; Fig. 3). Compared with control, the HC increased S-iCa ($P=0·001$, contrast analysis) more efficiently than did the AC ($P=0·02$, contrast analysis). The S-iCa increased significantly when the total Ca intake increased from 1080 mg to 1680 mg ($P=0·03$, contrast analysis). In addition, the S-iCa concentration dropped after 4 h from commencement of the study on the control day, while the concentration

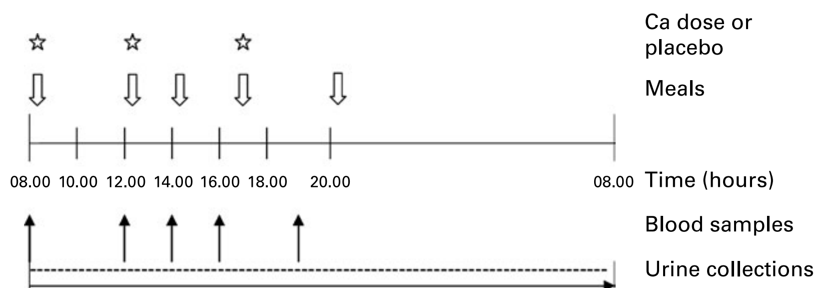

Fig. 2. Study design.

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Table 2. Total calcium intake of study subjects (*n* 12) and calcium:phosphorus ratios on study days

Ca dose (mg)	Total Ca intake (Ca dose + dietary Ca) (mg)	Ca:P ratio (mg:mg)
0 (control)	480*	0·26
600	1080*	0·58
1200	1680*	0·91

* Intakes of P and Ca from study day meals were 1850 mg and 480 mg, respectively.

remained nearly constant on both the AC and HC days. However, the total serum Ca concentration did not differ significantly between study sessions (*P*=0·35, ANOVA) (Table 3). No significant change was observed in S-Pi concentration due to increasing Ca doses (*P*=0·6, ANOVA) (Table 3). However, the S-Pi increased above the normal reference limit (1·4 mmol/l) in eight of the twelve subjects on the control day, in six of the twelve subjects on the AC day and in four of the twelve subjects on the HC day.

The S-PTH concentration decreased in response to the Ca doses (*P*<0·001, ANOVA) (Fig. 4). Contrast analysis revealed that the increase was equally significant on the AC and HC days (*P*=0·001), though the mean percentage decrease of S-PTH was higher with the HC than the AC days at all time points. On the control day, when the Ca intake was low, six of the twelve subjects had S-PTH values above the upper reference limit (>65 ng/l). Furthermore, on the control day, changes in S-PTH differed from both the AC and the HC days between 14.00 hours and 19.00 hours. While the concentration almost returned to the baseline value at 19.00 hours in the AC and HC sessions, the S-PTH value continued to increase on the control day. The maximum difference in the mean S-PTH values were 2 h after the last Ca dose (at 19.00 hours) being 34 % (*P*<0·001, AC, contrast analysis) and 40 % (*P*<0·001, HC, contrast analysis) lower than the S-PTH at 19.00 hours in the control day.

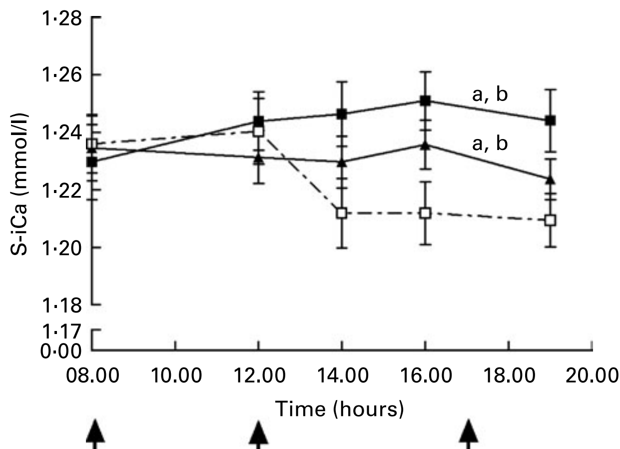

Fig. 3. Change in serum ionized calcium (S-iCa) concentrations during experiment days: control day (Ca intake 480 mg/d, -□-), 600 mg Ca dose (Ca intake 1080 mg/d, -▲-) and 1200 mg Ca dose (Ca intake 1680 mg/d, -■-). Values are means with their standard errors indicated by vertical bars. ↑, Ca administration times. [a] *P*<0·05 by ANOVA, repeated measure design. Mean values were significantly different from those of the control day (by contrast analysis): [b] *P*<0·05.

The 24-h urinary phosphate decreased in relation to the Ca doses (*P*=0·005, ANOVA) (Fig. 5). HC decreased phosphate excretion more efficiently (*P*=0·002, contrast analysis) than AC (*P*=0·089, contrast analysis). The 24-h urinary-Ca excretion significantly increased with the Ca doses (*P*=0·004, ANOVA) (Fig. 5). Urinary Ca increased by 38 % with AC (*P*=0·12, contrast analysis) and by 58 % with HC (*P*=0·001, contrast analysis).

The bone formation marker, S-BALP, did not differ significantly from the control day (*P*=0·4, ANOVA), although there was a trend to decreased bone formation in the HC day compared with the control day (Fig. 6). By contrast, the bone resorption marker, the 24-h excretion of U-NTx corrected for creatinine excretion (U-NTx:urinary creatinine), was affected by the Ca doses (*P*=0·008, ANOVA) (Fig. 6). The excretion of U-NTx diminished significantly with both Ca supplement doses (*P*=0·009 and *P*=0·02 for AC and HC, respectively, contrast analysis) being 14 % lower in the AC day, and 17 % lower in the HC day than in the control day.

All subject's blood samples consistently showed serum creatinine values within the normal reference range (60–115 μmol/l). Serum creatinine analysis was performed to ensure that none of the subjects had kidney disease, which could affect study results.

Discussion

The findings of the present study support our hypothesis that high Ca intake may not be sufficient to overcome adverse effects of high dietary P intake on Ca and bone metabolism within 24 h. Oral Ca supplement doses (600 and 1200 mg) suppressed both S-PTH and bone resorption (U-NTx) in a dose-dependent manner. To our knowledge, we demonstrated for the first time with this kind of study design the decrease in S-PTH concentration. However, even high Ca intake (in total 1680 mg/d) could not change the bone formation activity, which has been demonstrated to decrease due to high P intake in healthy females[4,9]. It is noteworthy that the P intake (1850 mg/d) corresponds to the average estimated dietary intake in Western countries and all ingested P origins in normal foods. The intake of Ca (1080 and 1680 mg/d), in turn, is beyond the reach of Western diets in many countries[24–28].

PTH is a major regulator of Ca and bone metabolism, and continuous excessive PTH secretion increases bone turnover[35,36]. Dietary P has been found to increase S-PTH levels, by decreasing S-iCa concentration[37] and by directly affecting PTH secretion[38], probably through the Na/Pi co-transporter in the parathyroid glands[39]. While P intake increases S-PTH concentration, Ca administration has been demonstrated to decrease S-PTH concentration in young adults[40,41]and the elderly[42] through an increase in S-iCa[37]. Our results showed S-PTH concentration to decrease with increasing Ca intake, indicating that Ca supplementation can reduce the rise in PTH levels, induced by higher dietary P intake. Furthermore, S-PTH concentration was above the upper reference limit (>65 ng/l) in 50 % of subjects on the control day (Ca 480 mg, P 1850 mg), suggesting adverse effects of the low dietary Ca:P ratio on S-PTH. In our recent controlled study in healthy females, we demonstrated that S-PTH increased in a dose-dependent manner with P intake[9]. These results are in accord with several studies in animals[7]

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Table 3. Values of calcium and phosphorus metabolism markers in serum at five time points
(Values are means with their standard errors)

	Sampling time										
	08.00 hours		12.00 hours		14.00 hours		16.00 hours		19.00 hours		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*-value
Serum Ca (S-Ca) (mmol/l)											
Control (C)	2·52	0·04	2·49	0·04	2·47	0·04	2·48	0·03	2·44	0·04	
600 mg Ca dose (AC)	2·48	0·03	2·53	0·05	2·55	0·04	2·52	0·04	2·52	0·03	NS
1200 mg Ca dose (HC)	2·59	0·05	2·53	0·02	2·54	0·03	2·55	0·03	2·53	0·03	NS
Serum P (S-Pi) (mmol/l)											
Control (C)	1·31	0·06	1·34	0·05	1·42	0·08	1·44	0·07	1·40	0·08	
600 mg Ca dose (AC)	1·31	0·06	1·34	0·05	1·40	0·05	1·43	0·05	1·43	0·07	NS
1200 mg Ca dose (HC)	1·28	0·05	1·34	0·05	1·35	0·06	1·41	0·06	1·36	0·05	NS

NS, not significant by ANOVA, repeated measures design.

and human subjects[4–6,43–45]. In the long run, high dietary P intake can lead to secondary hyperparathyroidism, especially when the dietary Ca intake is inadequate[7].

Ca supplementation decreased bone resorption, as indicated by the excretion of U-NTx:urinary creatinine. In earlier Ca administration studies, increased Ca intake was found to decrease bone resorption in young adults[40,41]and male athletes[46], while the effects of P on bone metabolism have been demonstrated to be just the opposite in P studies[4,9]and a study with high P–low Ca diets[5]. In our recent P study, we noted that bone resorption (U-NTx:urinary creatinine) increased when P intake was high (1995 mg/d) and Ca intake was very low (250 mg/d)[9]. In the present study, the decrease in bone resorption indicates that Ca supplementation diminished the effects of P intake higher than the recommended[8]. In the study of Grimm *et al.*[47] with ten healthy young females, the intake of P (3008 mg) increased the excretion of urinary pyridinoline and deoxypyridinoline by over 80 %, despite Ca intake being very high (1995 mg). However, the increase was not statistically significant due to the large standard deviation and the low number of subjects.

Although bone resorption decreased, we found no significant changes in bone formation, as indicated by S-BALP activity. In earlier studies, the effects of P intake on bone

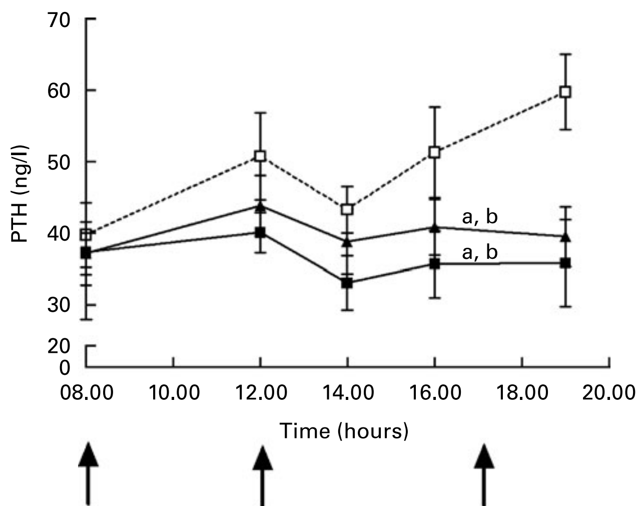

Fig. 4. Change in serum parathyroid hormone (PTH) concentrations during experiment days: control day (Ca intake 480 mg/d, -□-), 600 mg Ca dose (Ca intake 1080 mg/d, -▲-) and 1200 mg Ca dose (Ca intake 1680 mg/d, -■-). Values are means with their standard errors indicated by vertical bars. ↑, Ca administration times. [a] $P<0·05$ by ANOVA, repeated measure design. Mean values were significantly different from those of the control day (by contrast analysis): [b] $P<0·05$.

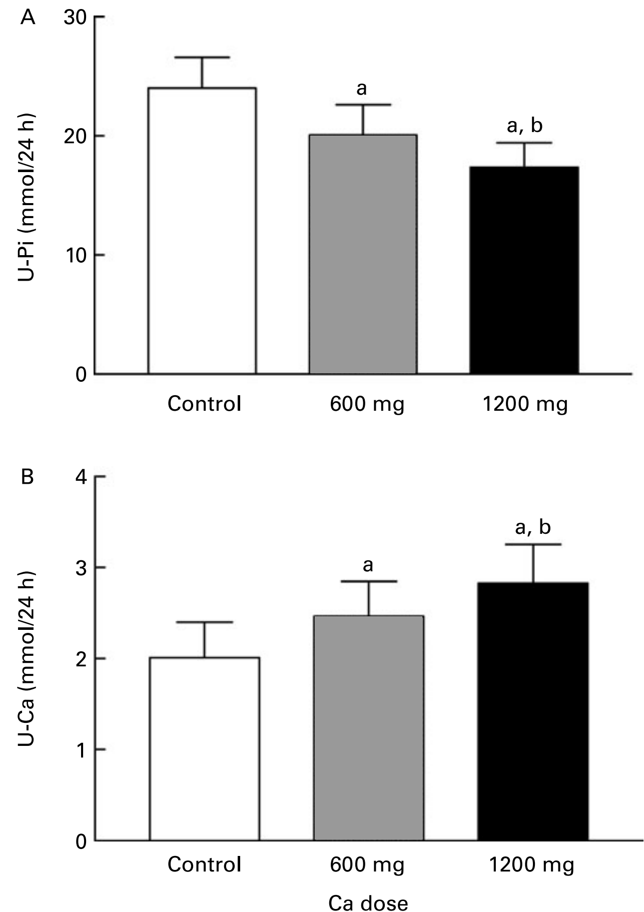

Fig. 5. The 24-h urinary phosphate (U-Pi; A) and urinary calcium (U-Ca; B) excretion during experiment days:. Values are means with their standard errors indicated by vertical bars. [a] $P<0·05$ by ANOVA, repeated measure design. Mean values were significantly different from those of the control day (by contrast analysis): [b] $P<0·05$. U-Pi 1 mmol/24 h = 30·93 mg/24 h, U-Ca 1 mmol/24 h = 40·08 mg/24 h.

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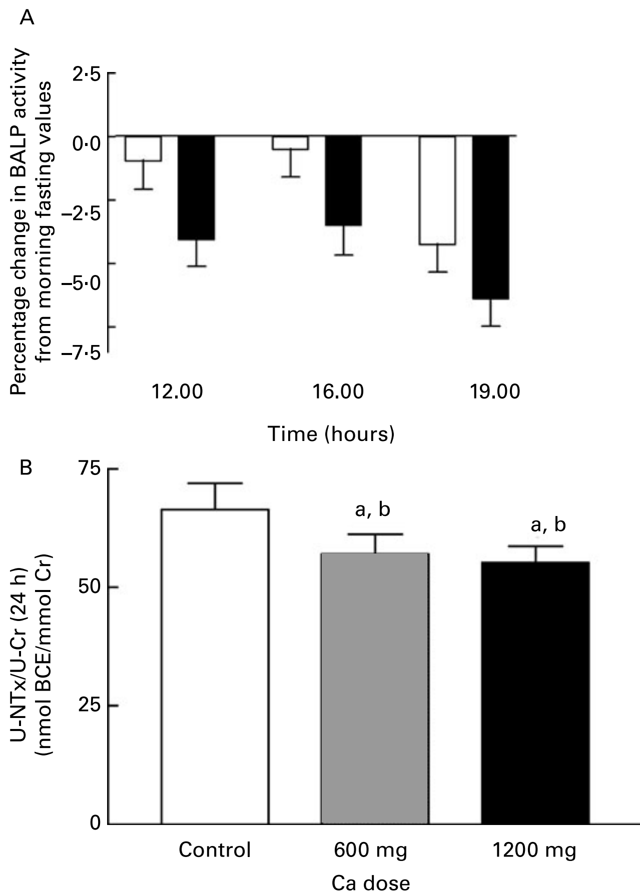

Fig. 6. Change in serum bone-specific alkaline phosphatase (BALP) activity from morning fasting values (A) and the 24-h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion (NTx:Cr) (B) during experiment days: control day (□), 600 mg Ca dose (▒) and 1200 mg Ca dose (■). Values are means with their standard errors indicated by vertical bars. [a] $P<0·05$ by ANOVA, repeated measure design. Mean values were significantly different from those of the control day (by contrast analysis) [b] $P<0·05$. BCE, bone collagen equivelents.

formation markers have been contradictory, with bone formation markers showing a decrease (S-BALP, serum procollagen type I carboxyterminal peptide, osteocalcin)[4,47], an increase (osteocalcin)[45] or no change (osteocalcin)[6]. Previous studies examining the effects of Ca on bone formation have also yielded inconsistent findings[40]. In our recent short-term study, we found that S-BALP activity decreased with increasing P doses[9]. In the present work the P intake was maintained at the same level (1850 mg/d) on each experiment day, and the effect of at this level of P intake on bone formation was presumably seen as a constant bone formation rate despite the varying dietary Ca intake. Thus, one possible explanation for our present findings is that high Ca intake cannot counteract the effects of P intakes higher than recommended[8], even though Ca supplementation seems to decrease bone resorption. If this interpretation is correct the effects of the increasing intake of dietary P on bone may be as alarming as previously summarized by Uribarri and Calvo[16] as well as Sax[48]. One may question whether we were able to detect changes in bone formation by measuring BALP activity since the half-life of BALP is 1–2 d[49]. However, one report indicates that BALP activity declines just 1 h after oral administration of 1-α-hydroxyvitamin D in peritoneal dialysis patients[50]. Furthermore, in physiological situations, PTH infusion can decrease BALP activity within 12 h[51].

S-iCa concentration increased in a dose-dependent manner with increasing Ca doses, but the increase was not reflected in total serum Ca concentration. In previous studies, a high intake of P decreased S-iCa concentration in females[4–6,9]and males[52]. However, Grimm *et al.*[47] reported that S-iCa concentration did not change even with 3000 mg P, probably due to the very high Ca content (1995 mg) of the diet. The mechanism underlying the impact of P on S-iCa remains unknown. In a Ca administration study, a Ca load as small as 250 mg was demonstrated to increase S-iCa[40]. In the present study, the lower the Ca:P ratio, the lower the S-iCa concentration and the higher the S-PTH concentration. However, this is hardly explained by only changes in Ca intake. Until recently, PTH secretion was believed to be mainly regulated by changes in S-iCa and 1,25-dihydroxyvitamin D, but there is evidence, which indicates that P *per se* increases PTH secretion[38].

We found no significant differences in S-Pi concentration, which was understandable as the P intake was constant on each study day. However S-Pi was higher than the normal reference limit (1·4 mmol/l) in several study subjects on the control day and on both AC and HC days. The lower the Ca intake, the more often S-Pi exceeded the upper reference limit. These findings suggest that P absorption diminished with increasing Ca intake but insufficiently to prevent excessive S-Pi concentrations. In studies where Ca intake has been adequate (1000 mg) or high (1995 mg), high P intake has not increased S-Pi concentration significantly[47,53]. This is probably due to diminished P absorption because of the formation of calcium phosphate complex in the gut. However, contradictory reports exist of the effects of P on Ca absorption[54–56], and relatively few studies are available. Spencer *et al.*[57] found that 800–2000 mg P doses did not affect Ca absorption in adults. Ca, on the other hand, has been shown to decrease P absorption[58].

S-Pi is mainly controlled by changes in urinary phosphate excretion[59,60]. Oral Ca intake ($CaCO_3$) has been demonstrated to diminish phosphate excretion[61]. This is due to increased S-iCa concentration, which in turns decreases S-PTH concentration and leads to lower phosphate excretion, even without any alterations in P intake[61]. In this study, we found that urinary phosphate excretion decreased with increasing Ca intake. The influence of Ca intake was also seen in the excretion of Ca, which increased in a dose-dependent manner. Because P intake was maintained at exactly the same level for all study day, the dose–response effect of Ca on phosphate and Ca excretion could be determined. Due to the short period of the study, we did not collect faecal samples, and thus, could not detect loss of Ca and P in faeces.

In summary, when P intake is above the dietary guidelines (700 mg/d)[8], oral Ca intake decreases S-PTH concentration and bone resorption which both have been induced by increased P intake[9]. Dietary P dose-dependently acutely decreases bone formation[9], but we found that in the present acute study even high Ca intake (in total 1680 mg/d) did not counteract this effect of dietary P intake, as indicated by unchanged bone formation activity. Our results suggest that higher doses of Ca than those used in our study are needed to prevent the effect of higher P intake, although lower doses also offer several

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favourable effects. Our findings further indicate that concerns regarding P consumption, especially in the Western world, are warranted. Future challenging tasks are to update the food composition tables and to determine the actual P contents of foods, including phosphate-containing food additives. Moreover, this study suggests that it is not only the Ca intake but also the dietary Ca:P ratio that matters. Additional studies are needed to confirm whether these findings persist over longer periods of time. Attention should be focused on decreasing P intake, as in many countries the recommended Ca intake level seems to be very difficult to achieve.

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