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High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females

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Ca and P are both essential nutrients for bone and are known to affect one of the most important regulators of bone metabolism, parathyroid hormone (PTH). Too ample a P intake, typical of Western diets, could be deleterious to bone through the increased PTH secretion. Few controlled dose–response studies are available on the effects of high P intake in man. We studied the short-term effects of four P doses on Ca and bone metabolism in fourteen healthy women, 20–28 years of age, who were randomized to four controlled study days; thus each study subject served as her own control. P supplement doses of 0 (placebo), 250, 750 or 1500 mg were taken, divided into three doses during the study day. The meals served were exactly the same during each study day and provided 495 mg P and 250 mg Ca. The P doses affected the serum PTH (S-PTH) in a dose-dependent manner ($P=0{\cdot}0005$). There was a decrease in serum ionized Ca concentration only in the highest P dose ($P=0{\cdot}004$). The marker of bone formation, bone-specific alkaline phosphatase, decreased ($P=0{\cdot}05$) and the bone resorption marker, N-terminal telopeptide of collagen type I, increased in response to the P doses ($P=0{\cdot}05$). This controlled dose–response study showed that P has a dose-dependent effect on S-PTH and increases PTH secretion significantly when Ca intake is low. Acutely high P intake adversely affects bone metabolism by decreasing bone formation and increasing bone resorption, as indicated by the bone metabolism markers.

Parathyroid hormone: Bone metabolism: Phosphorus intake: Dose–response study

Osteoporosis is considered a major public health problem in the developed countries and it has been predicted that the future number of hip fractures will increase (Gillet & Reginster, 1999; Kannus *et al.* 1999). Although P is an essential mineral for bone, it is consumed in amounts that are too high for optimal bone health, compared with the amount of Ca intake (Calvo, 1993; Calvo & Park, 1996; Sax, 2001).

Unlike Ca (Weaver & Heaney, 1999), dietary P is well absorbed (55–80 %) from the intestine (Heaney, 2004) and it increases serum phosphate (S-Pi) concentration within hours (Kärkkäinen & Lamberg-Allardt, 1996). The effect of an increased dietary phosphorus load on S-Pi concentration is mainly controlled by changes in urinary excretion of phosphate. High S-Pi and serum parathyroid hormone (S-PTH) concentrations increase urinary phosphate (U-Pi) excretion by down-regulating sodium–phosphate cotransporters in the kidneys (Takeda *et al.* 1999).

High dietary phosphorus intake is typical in countries where processed foods with food additives are frequently consumed (Calvo, 1994). Although some foods rich in phosphorus are also good sources of calcium, e.g. milk products, many others contain very little calcium. During the latest decades the use of phosphate-containing food additives has increased in many countries, e.g. in the USA (Calvo & Park, 1996); such additives may increase the phosphorus intake by as much as 1 g/d (Uribarri & Calvo, 2003). An increasing problem is that nutrition composition tables usually do not include the phosphorus from these additives, which leads to underestimation of the phosphorus intake (Uribarri & Calvo, 2003). In Finland the mean phosphorus intake of women is about 1300 mg and that of men 1800 mg (Männistö *et al.* 2003), i.e. in women the average intake exceeds the dietary reference intake (700 mg/d) (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Research Council, 1997), by almost 2-fold and in men by 3-fold. Phosphorus intakes are also higher than recommended in other European countries (Gregory *et al.* 1990; Grimm *et al.* 2001; Gronowska-Senger & Kotanska, 2004). Nevertheless, it has been suggested that older women with osteoporosis should be given phosphorus supplements, because their dietary phosphorus intake is too low due to frequent calcium supplementation ,which results in decreased phosphorus absorption (Heaney & Nordin, 2002).

In many Western communities phosphorus intake is high whereas calcium intake might be low. The overall trend in food consumption in Europe (Urho & Hasunen, 1999; Comité de Nutrición de la Asociación Española de Pediatría,

Abbreviations: AUC, area under the curve; BALP, bone-specific alkaline phosphatase; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; S-iCa, serum ionized Ca; $S\text{-}1,25(OH)_2D$, serum 1,25-dihydroxyvitamin D; S-Pi, serum phosphate; S-PTH, serum parathyroid hormone; U-Ca, urinary Ca; U-Cr, urinary creatinine; U-NTx, urinary N-terminal telopeptide of collagen type I; U-Pi, urinary phosphate.

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2003) as well as in the USA (Calvo & Park, 1996; Harnack *et al.* 1999; Nielsen & Popkin, 2004) is to drink less milk and more phosphoric acid-containing soft drinks. In fact, it was reported that consumption of cola beverages may predict a higher risk of fracture in girls (Wyshak, 2000) and result in development of higher S-PTH concentration and hypocalcaemia in postmenopausal women (Fernando *et al.* 1999). These types of dietary habit may lead to the lower dietary Ca:P ratios that were recently observed in many countries (Brot *et al.* 1999; Chwojnowska *et al.* 2002; Takeda *et al.* 2002; Männistö *et al.* 2003). Furthermore, recent evidence from Poland revealed that among 10 % of young girls and boys, the dietary Ca:P ratio was lower than 0·25 (Chwojnowska *et al.* 2002). These results support previous findings among young women in the USA (Calvo *et al.* 1990).

A diet high in phosphorus resulted in an increase in S-PTH secretion in animals (Calvo & Park, 1996) as well as human subjects in different kinds of study design (Calvo *et al.* 1988, 1990; Kärkkäinen & Lamberg-Allardt, 1996). Thus, in the long run high dietary phosphorus intake could lead to secondary hyperparathyroidism and lower bone mineral density if the dietary calcium intake is inadequate. Katsumata *et al.* (2005) and Huttunen *et al.* (2005) demonstrated recently that diets high in phosphorus resulted in secondary hyperparathyroidism and bone loss in rats. In fact, evidence is available from an epidemiological cross-sectional study in human subjects that phosphorus intakes greater than recommended amounts are negatively and independently associated with lower amounts of bone mass (Metz *et al.* 1993).

The primary aim of the present study was to investigate in a controlled situation, over the entire day, how four dietary phosphorus doses, obtainable in Western diets, acutely affect the markers of calcium and bone metabolism. Calcium intake was kept as low as possible to avoid the effects of calcium on parathyroid hormone (PTH) secretion. The secondary aim was to examine whether the effects of phosphorus were dose-dependent. We confined the present study to women because the incidence of osteoporosis is more common among women than men (Kannus *et al.* 1999).

Subjects and methods

Subjects

Fifteen women, 20–28 years of age, were recruited among university students. All subjects gave their informed consent to the procedures, which were in accord with the Helsinki Declaration. The Helsinki University Ethics Committee approved the protocol. The exclusion criteria were illnesses and medications (other than oral contraceptives) affecting bone and mineral metabolism. Seven of the subjects used oral contraceptives. Fourteen women completed the study. One woman interrupted the study because of severe headache. The basic characteristics of the subjects are presented in Table 1.

Table 1. Basic characteristics of the study subjects (*n* 14)

Variable	Mean	Range
Age (years)	24	20–28
Weight (kg)	59	48–70
Height (cm)	169	160–178
BMI (kg/m^2)	20·9	17·8–22·9
Habitual dietary Ca intake (mg/d)	1134	570–1910
Habitual dietary P intake (mg/d)	1501	1006–2022
Habitual dietary Ca:P ratio	0·74	0·55–0·94
Habitual dietary energy intake (MJ)	7·9	5·7–14·6

For details of subjects and procedures, see this page.

Study design

The subjects attended four 24 h sessions during a 1-month period with 1 week between the sessions. The study design is presented in Fig. 1. Each study subject served as her own control. The subjects were given, in randomized session order, 0 (placebo), 250, 750 or 1500 mg phosphorus as commonly used phosphate additive in the food industry (mixture of disodium phosphate and trisodium phosphate (P content 25 %); Six Oy, Helsinki, Finland) in 1000 ml berry juice during the sessions. The juice with or without phosphorus was served in three equal-sized separate 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 16.00 hours (with a snack after the fourth blood sample). The phosphate additive, which we used, contained 31·2 % Na (312, 936 and 1872 mg as Na, respectively). In our earlier study we did not find 1407 mg Na as Nacl to affect the serum intact PTH concentration during 6 h; thus the possibility that the effect on PTH secretion is due to Na and not due to phosphorus is ruled out (Kärkkäinen & Lamberg-Allardt, 1996).

The meals served were identical for all subjects on each study day. Breakfast was served at 08.00 hours, lunch at 12.00 hours, dinner at 17.00 hours and supper at 20.00 hours; snacks were served at 14.00 and 16.00 hours. The subjects were not allowed to eat anything else during the study day. Water was provided *ad libitum*. The meals provided a total energy content of 8·4 MJ (2000 kcal), with a calcium content of 250 mg and phosphorus content of 495 mg/d, by calculation. The meals included normal foods bought from Finnish grocery stores. Thus, the present study design included phosphorus intakes of 495 mg (placebo) and 745 mg (250 mg phosphorus dose); with phosphorus intake at a recommended level (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Research Council, 1997). The phosphorus intake of 1245 mg (750 mg phosphorus dose) corresponds to the mean phosphorus intake of Finnish females (Männistö *et al.* 2003), while the phosphorus intake of 1995 mg (1500 mg phosphorus dose) typifies the mean phosphorus intake of Finnish males (Männistö *et al.* 2003). The total phosphorus intakes and dietary Ca:P ratios of subjects throughout the study sessions are presented in Table 2.

The first blood samples were taken anaerobically at 08.00 hours after an overnight fast at each study session and the following samples at 12.00, 14.00, 16.00, 18.00 and 08.00 hours the following morning. The 24 h urine collections were started at 08.00 hours at each study session and ended at 08.00 hours the following morning. The urine and separated serum samples were stored at −20°C until analysis. The sampling procedure is presented in Fig. 1.

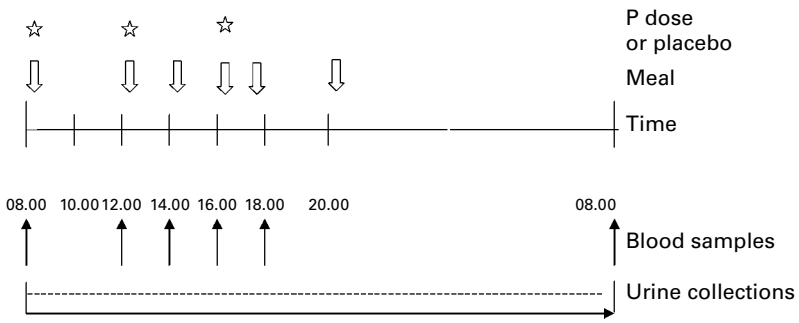

Fig. 1. The design of the study days. For details of subjects and procedures, see p. 546. ☆,P or placebo administration times; ⇓,meal times; ↑,blood sampling times; →, 24-h urine collection.

Habitual dietary intake of subjects

To estimate the energy and the calcium and phosphorus intakes, the subjects were instructed in how to keep a 4 d food record that included three weekdays and one weekend day. The subjects were instructed to maintain their usual food intake while recording and to record all foods and beverages immediately after consumption. The subjects' habitual dietary intake was calculated, using a computer-based program (Flamingo, version 0.5.6; Dipper software, Helsinki, Finland) based on the food composition database (Fineli) of the Finnish National Public Health Institution. The basic characteristics of the subjects' usual dietary intake are shown in Table 1.

Laboratory methods

The serum ionized Ca (S-iCa) concentration was analysed within 2 h of the sample collection from anaerobically handled serum samples with an ion-selective analyser (Microlyte 6; Thermo Electron Corporation, Vantaa, Finland). The intra-assay CV was 1·6 % for S-iCa. The S-Pi, and urinary Ca (U-Ca), U-Pi and urinary creatinine (U-Cr) were analysed spectrometrically with an autoanalyser (Konelab 20 automatic analyser, Thermo Electron Corporation). The intra- and inter-assay CV for these analyses were <2 % and $<3{\cdot}5$ %, respectively. The serum intact PTH concentration was determined with an Allegro intact PTH kit (Nichols Institute, San Juan Capistrano, CA, USA); the intra- and inter-assay CV were 1 % and 4 %, respectively. Bone-specific alkaline phosphatase (BALP) was analysed with an enzyme immunoassay (Metra Biosystems, Palo Alto, CA, USA). The BALP analyses were made from the control (placebo) and the 750 and 1500 mg phosphorus dose sessions from the 0, 8, 10 and 24 h samples; the intra- and inter-assay CV were 4 % and 6 %, respectively. The concentration of urinary N-terminal telopeptide of collagen I (U-NTx) was determined with an ELISA Osteomark NTx test (Ostex International Inc., Seattle, WA, USA) from the control (placebo) and the 750 and 1500 mg phosphorus dose sessions; the intra- and inter-assay CV were 9·7 % and 11·8 %, respectively. Serum 1,25-dihydroxyvitamin D (S-1,25$(OH)_2$D) was analysed with an IDS RIA kit (Immunodiagnostic Systems Ltd, Boldon, UK) from the control and the 1500 mg phosphorus dose sessions from the 0, 8, 10 and 24 h samples; the intra- and inter-assay CV were 9 % and 10 %, respectively. All samples from the same person were analysed in the same assay in a randomized order.

Table 2. Total P intake of the study subjects (*n* 14) and Ca:P ratios in study days*

P dose (mg)	Total P intake (P dose + dietary P) (mg)†	Ca:P ratio
0 (placebo)	495‡	0·51
250	745§	0·34
750	1245‖	0·20
1500	1995¶	0·13

* For details of subjects and procedures, see p. 546.
† Intake of P and Ca from study day meals 495 and 250 mg, respectively.
‡ Lower intake than recommended P intake (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Research Council, 1997).
§ Intake equal to the recommended P intake (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Research Council, 1997).
‖ Intake same as mean dietary P intake of Finnish females (Männistö *et al.* 2003).
¶ Intake same as mean dietary P intake of Finnish males (Männistö *et al.* 2003).

Statistical analysis

The data are expressed as means with their standard errors. The variables were tested for normality and logarithmic transformations were used to normalize non-normal distributions. For serum variables the area under the curve (AUC) for the difference from the morning fasting value was calculated. Although in the figures the curves are presented as percentages from the morning fasting value, the original values are used in the statistical calculations. ANOVA with repeated measures was used to compare the study periods. If the sphericity assumption was violated, Hyunh-Feldt adjustment was used. The effects of the phosphorus doses were compared with the control session with contrast analysis. All the analyses were performed with the SPSS statistical package, version 10.0 (SPSS Inc., Chicago, IL, USA) in a Windows environment. We regarded $P<0{\cdot}05$ as significant.

Results

Baseline characteristics

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

Markers of calcium and bone metabolism

There was a significant dose–response relationship in the S-Pi concentration in relation to the phosphorus doses ($P=0{\cdot}0005$, ANOVA; Fig. 2). The increase was already significant with the 250 mg phosphorus dose ($P=0{\cdot}04$, contrast analysis), and more profound after the two highest doses ($P=0{\cdot}002$ and $P=0{\cdot}0005$, for the 750 and 1500 mg phosphorus doses, respectively, contrast analysis). The S-Pi level increased above the normal reference limit (1·4 mmol/l) in four out of fourteen subjects with the 750 mg phosphorus dose and in seven out of fourteen subjects with the 1500 mg phosphorus dose. The S-Pi concentration tended to be higher on the morning following the 1500 mg phosphorus dose ($P=0{\cdot}09$) than in the morning following the fasting sample of the previous day. The S-iCa concentration declined (Fig. 2) in response to the phosphorus intake ($P=0{\cdot}0005$, ANOVA) but was significant only after the 1500 mg phosphorus dose ($P=0{\cdot}004$, contrast analysis). The S-iCa was still decreased after the 1500 mg phosphorus dose on the following morning ($P=0{\cdot}004$) compared with the morning fasting value of that study session.

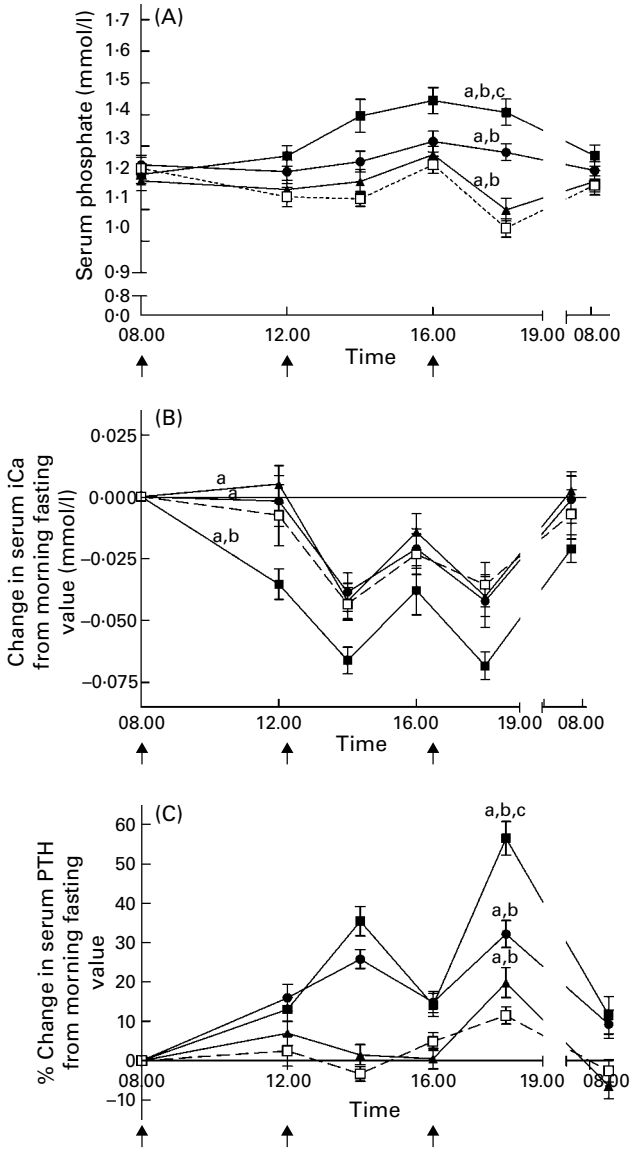

Fig. 2. Change in (A), serum phosphate; (B), serum ionized Ca (iCa); and (C) serum parathyroid hormone (PTH) concentrations during the study days: control day (–□–), 250 mg P dose (–▲–), 750 mg P dose (–●–) and 1500 mg P dose (–■–). For details of subjects and procedures, see p. 546. Values are means with their standard errors depicted by vertical bars. [a]$P<0{\cdot}05$ (by ANOVA, repeated measures design). [b,c] Mean values were significantly different from those of the control day (by contrast analysis): [b]$P<0{\cdot}05$; [c]$P<0{\cdot}001$. ↑, P administration times.

As shown in Fig. 2, the S-PTH concentration increased in a dose-dependent manner in response to the phosphorus intake ($P=0{\cdot}0005$, ANOVA). Contrast analysis showed that these increments were significant with all doses ($P=0{\cdot}03$, $P=0{\cdot}002$ and $P=0{\cdot}0005$ with the 250, 750 and 1500 mg phosphorus doses, respectively). The maximum difference in S-PTH from the morning fasting level was 2 h after the last dose of phosphorus (at 18.00 hours), being 20 % ($P=0{\cdot}3$, 250 mg phosphorus dose), 32 % ($P=0{\cdot}009$, 750 mg dose) and 57 % ($P=0{\cdot}006$, 1500 mg dose) above the morning fasting value. With the two highest phosphorus doses these increases were significant ($P=0{\cdot}03$, $P=0{\cdot}0005$, respectively, contrast analysis) compared with the 12 % increment during the control session. Of the fourteen subjects, three persons during the 1500 mg phosphorus dose session and one person during the 750 mg session had S-PTH values above the upper reference limit (>65 ng/l).

The U-Pi excretion (Fig. 3) increased in a dose-dependent manner ($P=0{\cdot}0005$, ANOVA) with the increasing phosphorus doses. The U-Pi excretion increased by 27 % with the 250 mg phosphorus dose ($P=0{\cdot}08$, contrast analysis), 70 % with the 750 mg dose ($P=0{\cdot}0005$, contrast analysis) and 126 % with the 1500 mg dose ($P=0{\cdot}0005$, contrast analysis). The 24 h U-Ca excretion (Fig. 3) decreased significantly with increasing phosphate doses ($P=0{\cdot}0005$, ANOVA). The phosphorus doses of 750 and 1500 mg both decreased the U-Ca excretion significantly ($P=0{\cdot}0005$ and $P=0{\cdot}002$, respectively, contrast analysis) compared with the control session.

The AUC of the S-1,25$(OH)_2$D concentration did not change significantly in response to the 1500 mg phosphorus dose ($P=0{\cdot}2$, ANOVA; Fig. 4). However, the S-1,25$(OH)_2$D concentration was increased on the following morning (24 h) at the control session compared with the previous morning fasting value ($P=0{\cdot}05$), whereas at the 1500 mg phosphorus dose session there was no change ($P=0{\cdot}9$). The increase in S-PTH (AUC) correlated positively with the morning fasting S-1,25$(OH)_2$D concentration (r 0·57, $P=0{\cdot}035$).

There was a significant ($P=0{\cdot}009$, ANOVA) decline in serum BALP activity, a marker of bone formation after the 750 and 1500 mg phosphorus doses (Fig. 5). The 1500 mg phosphorus dose decreased serum BALP activity significantly ($P=0{\cdot}004$, contrast analysis) but with the dose of 750 mg the decline was not significant ($P=0{\cdot}75$, contrast analysis) compared with the control session. The 24 h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion (U-NTx/U-Cr) (Fig. 5) was affected by the phosphorus intake ($P=0{\cdot}048$, ANOVA). With the 1500 mg phosphorus dose U-NTx/U-Cr was 33 % ($P=0{\cdot}06$, contrast analysis) above the level of the control day. The U-NTx/U-Cr tended to correlate with the increase in S-Pi (AUC) (r 0·42, $P=0{\cdot}13$) but not with the increase in S-PTH (AUC) ($P=0{\cdot}6$).

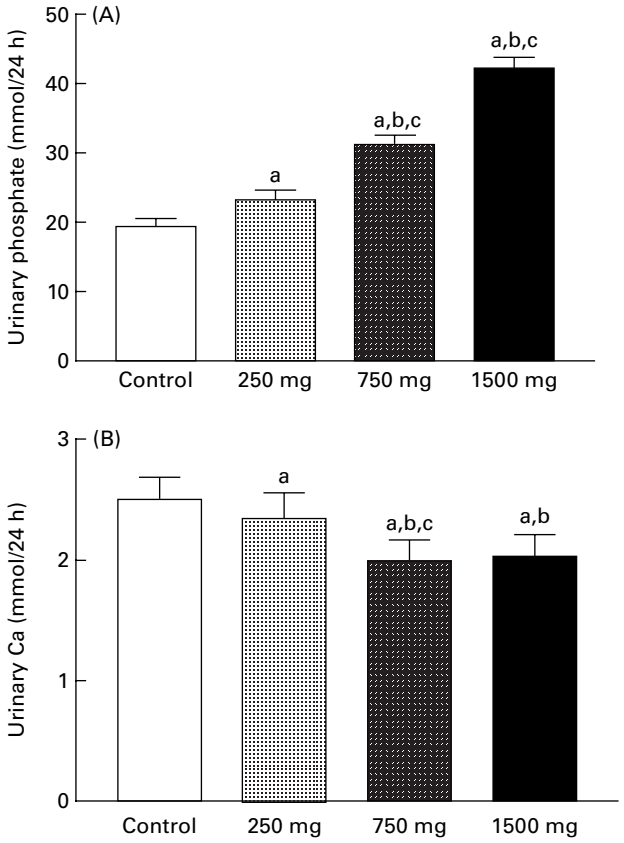

Fig. 3. The 24 h urinary phosphate (A) and Ca (B) excretion during the study days. For details of subjects and procedures, see p. 546. Values are means with their standard errors depicted by vertical bars. [a]$P<0{\cdot}05$ (by ANOVA, repeated measures design). [b,c] Mean values were significantly different from those of the control day (by contrast analysis): [b]$P<0{\cdot}05$; [c]$P<0{\cdot}001$.

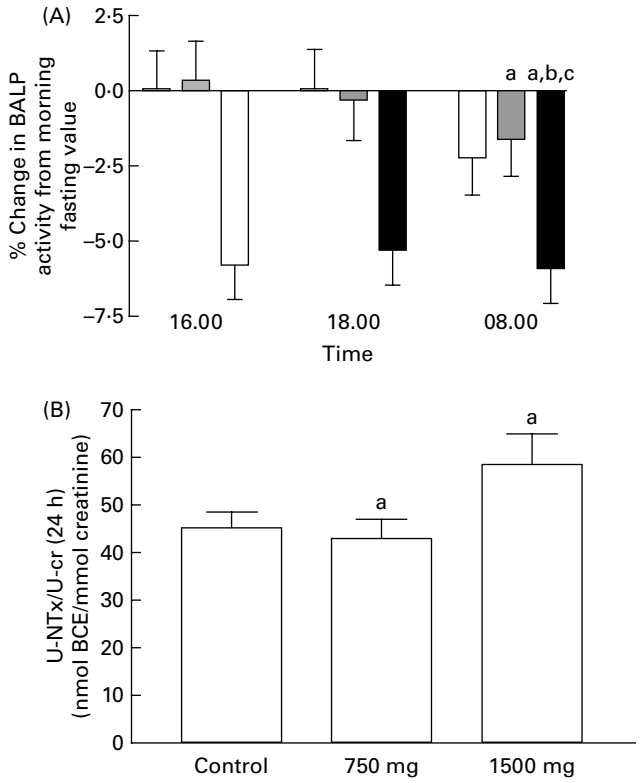

Fig. 5. (A), Change in serum bone-specific alkaline phosphatase (BALP) activity during the study days (□, control day; ■, 750 mg P dose; ■, 1500 mg P dose). (B), The 24 h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion (U-NTx/U-Cr) during the study days. For details of subjects and procedures, see p. 546. Values are means with their standard errors depicted by vertical bars. [a]$P<0{\cdot}05$ (by ANOVA, repeated measures design). [b] Mean value was significantly different from that of the control day (by contrast analysis): $P<0{\cdot}05$. BCE, bone collagen equivalents.

Discussion

In the present study we found for the first time in healthy human subjects a dose-dependent increase both in S-Pi and S-PTH concentrations due to increase in the dietary phosphorus intake. The high phosphorus load also affected the bone metabolism markers negatively. In the present study the intake of phosphorus (dietary + phosphorus dose) corresponding to the average estimated dietary intakes of females and males in Western countries increased the S-PTH concentration significantly. Furthermore, with the 1500 mg phosphorus dose three subjects out of fourteen and with the 750 mg dose one subject had S-PTH values above the higher reference limit of 65 ng/l, and with the two highest phosphorus doses S-PTH values were still elevated the following morning, indicating the presence of transient secondary hyperparathyroidism. It is important to note that phosphorus was ingested throughout the day, simulating the situation in which food with high phosphorus content is ingested. This implies that foods with high phosphorus content may cause transient hyperparathyroidism in healthy individuals at least when the calcium intake is low.

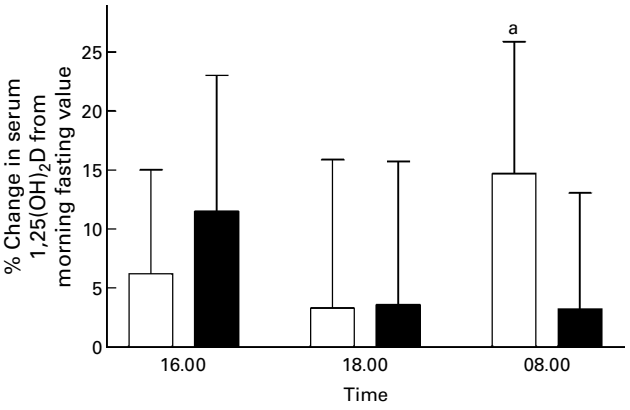

Fig. 4. Change in serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) during the study days: control day (□) and 1500 mg P dose (■). For details of subjects and procedures, see p. 546. Values are means with their standard errors depicted by vertical bars. [a] Mean values were significantly different from the morning fasting value of the control day: $P<0{\cdot}05$.

In our earlier study in human subjects we showed that 1500 mg phosphorus/d increases S-PTH secretion (Kärkkäinen & Lamberg-Allardt, 1996). The phosphorus intake of that study corresponds to the highest phosphorus dose in our present study. To our knowledge there are only two previous studies (Brixen *et al.* 1992; Whybro *et al.* 1998) concerning the dose–response effect of oral phosphorus loads in human subjects. In these studies the calcium intake was adequate or high (Whybro *et al.* 1998) or unknown (Brixen *et al.* 1992). In the 4-week intervention study in young men by Whybro

et al. (1998), phosphorus supplementation did not affect S-PTH, thus supporting the importance of adequate Ca:P ratios in the diet. The recommended optimal Ca:P ratio in the diet is 1·0 on a molar basis (Calvo & Park, 1996; Whybro *et al.* 1998), which corresponds to 1·3 on a mg basis. Understandably criticism was raised against the report *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Research Council (1997) (Sax, 2001). The report excluded several studies whose results supported the importance of the role played by the dietary Ca:P ratio in bone health.

In the present study we found a significant decline in serum BALP activity, indicating inhibition of bone formation due to high phosphorus intake. The present finding is in accordance with the results of two previous studies (Kärkkäinen & Lamberg-Allardt, 1996; Grimm *et al.* 2001). The results of the effects of phosphorus intake on bone formation and resorption markers published in earlier studies have been conflicting. In some studies, bone formation markers have either decreased (BALP, serum procollagen type I carboxyterminal peptide, osteocalcin; Kärkkäinen & Lamberg-Allardt, 1996; Grimm *et al.* 2001), increased (osteocalcin; Silverberg *et al.* 1986; Brixen *et al.* 1992) or shown no change (osteocalcin; Calvo *et al.* 1990). The differences in protocols, e.g. in calcium and phosphorus intakes, length of the study, as well as differences in the sensitivity of bone metabolism markers, probably explain the differences in the results. In the present study we chose BALP as a bone formation marker as we could demonstrate a rapid decrease in BALP activity induced by the phosphorus load in our previous study (Kärkkäinen & Lamberg-Allardt, 1996) In fact, BALP activity has been demonstrated to decline just 1 h after oral administration of 1-α-hydroxyvitamin D in peritoneal dialysis patients (Joffe *et al.* 1994) and PTH infusion has been found to decrease BALP activity within 12 h in physiological situations (Hodsman *et al.* 1993).

While bone formation decreased, we also demonstrated that the marker of bone resorption, U-NTx/U-Cr, increased due to phosphorus loading, indicating increased bone resorption during the high phosphorus intake. High dietary phosphorus increases S-PTH and it is well known that PTH increases bone resorption. In contrast, *in vitro* phosphorus decreases the activity of osteoclasts (Yates *et al.* 1991). In our previous study (Kärkkäinen & Lamberg-Allardt, 1996) we found no increase in the serum type I collagen c-terminal telopeptide or in the free form of urinary deoxypyridinoline/U-Cr excretion after a 1500 mg phosphorus dose. The discrepancy in our previous study could have been due to the fact that in some studies neither serum type I collagen c-terminal telopeptide (Garnero *et al.* 1994) nor the free form of urinary deoxypyridinoline/U-Cr (Rubinacci *et al.* 1999) were very sensitive markers of bone resorption. In different settings, the other markers of bone resorption such as urinary hydroxyproline either increased (Calvo et al. 1988) or showed no change (Silverberg *et al.* 1986).

In the present study there was a decrease in S-iCa only with the highest phosphorus dose. However, S-PTH also increased with the smaller phosphorus doses (250 and 750 mg) despite the lack of a decrease in S-iCa thus supporting the findings of previous *in vitro* studies in cell culture (Almaden *et al.* 1998) and with animals (Estepa *et al.* 1999). Furthermore, the dietary calcium intake was low throughout the present study, and it was demonstrated that even a calcium load as small as 250 mg increases S-iCa and decreases S-PTH concentrations (Kärkkäinen *et al.* 2001). Thus, the low calcium intake could not have been responsible for these low S-iCa values in the present study. In addition, since calcium metabolism is regulated by the interplay of hormones and mineral ions, it was believed until recently that PTH secretion was mainly regulated by changes in ionized Ca and in 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). However, there is evidence that phosphorus *per se* increases PTH secretion (Slatopolsky *et al.* 1996), probably through the Na–phosphate cotransporter in the parathyroid glands (Miyamoto *et al.* 1999).

S-Pi is mainly controlled by changes in U-Pi excretion (Murer *et al.* 2000). In the present study the S-Pi concentration increased in a dose-dependent manner in response to the phosphorus intake, thus leading to the dose-dependent increase in U-Pi excretion. It is known that PTH increases U-Pi and decreases U-Ca excretion. Recently, it was shown that both PTH and phosphorus intake itself down-regulate the Na–phosphate cotransporter in the kidneys (Takeda *et al.* 1999), thereby increasing U-Pi and decreasing U-Ca excretions. In the present study, U-Ca excretion decreased with all phosphorus doses, but the decrease was significant only with the two highest doses (750 and 1500 mg). However, the excretions with these two doses were similar, suggesting that the excretion might not diminish after a certain high dietary phosphorus intake but may remain at a certain level despite the higher S-Pi and S-PTH concentrations, thus resulting in unfavourable calcium balance.

The active vitamin D metabolite, $1,25(OH)_2D$, plays an important role in calcium metabolism, e.g. by increasing calcium absorption in the gut. The S-$1,25(OH)_2D$ concentration increases in response to a decrease in calcium intake (Dawson-Hughes *et al.* 1993), to a decrease in S-Ca and to high concentrations of S-PTH (Boden & Kaplan, 1990), and decreases in response to high dietary phosphorus intake (Portale *et al.* 1989). Furthermore, $1,25(OH)_2D$ increases the Na^+–Pi cotransporter mRNA in the parathyroids (Miyamoto *et al.* 1999), which could mediate the effects of extracellular phosphorus on the parathyroid glands. In the present study we noted an increase in S-$1,25(OH)_2D$ concentration after 24 h on the control day, when both the calcium and phosphorus intakes were low, as expected. However, in the present study a high phosphorus dose (1500 mg) did not change S-$1,25(OH)_2$-D concentration after 24 h despite a low calcium intake and an increase in S-PTH during that day. The present finding suggests that a normal increase in calcium absorption induced by an increase in $1,25(OH)_2D$ in consequence to a low calcium intake is inhibited by a high phosphorus intake. Thus, high phosphorus intake could decrease active calcium absorption in the long run (Portale *et al.* 1986). The present results are in accordance with a previous experimental study in animals (Martin-Malo *et al.* 1996) and in human subjects (Calvo *et al.* 1990; Grimm *et al.* 2001). However, in the study of Grimm *et al.* (2001) the calcium intake was high (2000 mg), thus counteracting the stimulus of PTH on 1-α-hydroxylase.

The results of the present study indicate that dietary phosphorus intake levels typical of Western diets acutely and negatively affect calcium and bone metabolism in healthy individuals, at least when dietary calcium intake is low. Due to increasing use of processed foods with phosphate-containing food additives in addition to an alarming rise in the incidence of osteoporosis (Gillet & Reginster, 1999; Kannus *et al.* 1999) and especially of type 2 diabetes (Laakso, 2005), a major cause of end-stage renal disease (Kramer, 2005), in which dietary phosphorus restriction is a part of the treatment, it is vitally important to focus concern on the dietary phosphorus intake and Ca:P ratios among Western populations. In addition, vascular calcification highly correlates with CVD mortality, especially among patients with end-stage renal disease and diabetes (Giachelli, 2004) and results from an *in vitro* study suggest that excess phosphorus intake may be involved in this vascular calcification process (Jono *et al.* 2000).

In conclusion, oral intake of phosphorus in doses comparable to normal dietary intakes with low calcium intake increased S-Pi and S-PTH in a dose-dependent manner in healthy young females. High, often habitual intake of phosphorus caused an increase in NTx, a marker of bone resorption, and a decrease in BALP, a marker of bone formation. The present results indicate that bone resorption increased whereas formation decreased, thus leading to an unfavourable imbalance in bone metabolism. Furthermore, high phosphorus intake inhibited the increase in S-1,25$(OH)_2$D in response to low dietary calcium intake. The present finding implies that the normal relationship between calcium intake and calcium absorption is disturbed in a diet high in phosphorus and low in calcium. Additional studies are needed to confirm whether the present findings persist over longer periods of time. In addition, controlled studies of different dietary calcium levels together with high phosphorus intake are needed to evaluate how much calcium should be consumed to gain protection against the harmful effects of the high phosphorus intakes typical of Western diets.

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