Habitual high phosphorus intakes and foods with phosphate additives negatively affect serum parathyroid hormone concentration: a cross-sectional study on healthy premenopausal women

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Abstract

Objective: Foods can contain natural phosphorus (NP) and phosphate-containing food additives (AP). The main objective of the present study was to investigate whether NP and AP of habitual diets differ in their effects on markers of Ca metabolism. We also investigated the impact of total habitual dietary P intake on markers of Ca metabolism.

Design: Cross-sectional study. Fasting blood samples were collected and participants kept a 4 d food record, from which dietary intake of total P and the consumption of NP (milk and cheese, excluding processed cheese) and AP (processed cheese) sources were calculated. Participants were divided into groups according to their NP- and AP-containing food consumption and into quartiles according to their total P intake.

Setting: Southern Finland.

Subjects: One hundred and forty-seven healthy premenopausal women aged 31–43 years.

Results: Relative to the lowest total dietary P quartile, mean serum parathyroid hormone (S-PTH) concentration was higher ($P = 0.048$, analysis of covariance (ANCOVA)) and the mean serum ionized Ca concentration lower ($P = 0.016$, ANCOVA) in the highest P intake quartile. Mean S-PTH concentrations were higher among participants who consumed processed cheese ($P = 0.027$, ANCOVA) and less milk and other cheese than processed cheese ($P = 0.030$, ANCOVA).

Conclusions: High total habitual dietary P intake affected S-PTH unfavourably. Furthermore, phosphate additives may have more harmful effects on bone than other P sources, as indicated by higher mean S-PTH concentration among participants who consumed AP-containing foods. Because of the high dietary P intake and current upward trend in consumption of processed foods in Western countries, these findings may have important public health implications.

P unlike Ca is abundant in many food sources, as foods can contain both natural P and phosphate-containing food additives. In many countries, P intake is two- to threefold higher[1–4] than the dietary reference intake for P (700 mg/d)[5], while Ca intake is often below recommended levels[6–9]. Such a combination, or even a high P intake alone, may be harmful to bone through increased parathyroid hormone (PTH) secretion[10–13].

PTH is a major regulator of Ca and bone metabolism, and continuous excessive PTH secretion increases bone turnover[14,15]. Dietary P has been found to increase serum PTH (S-PTH) concentration by decreasing serum ionized Ca (S-iCa) concentration[16] and by directly affecting PTH secretion[17], probably through the Na-dependent phosphate co-transporter in the parathyroid glands[18]. In contrast to continuous excessive PTH secretion, intermittent administration of PTH stimulates bone formation[19] and increases trabecular bone mass[20]. Therefore, *in vivo*, the combined effect of P and PTH on bone metabolism is complex and may vary from an acute situation to a long-term one.

P sources may differ in their effects on Ca and bone metabolism; a recent short-term controlled study revealed that P originating from phosphate additives alone

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increased S-PTH concentration more than P from cheese, meat and wholegrain products[21]. Intestinal absorption of P is efficient and absorption occurs by both passive and active transport, the latter being stimulated by 1,25-dihydroxyvitamin D[22]. The absorbability of P in Ca-containing dairy products is not well known; however, in renal disease Ca compounds are used to bind dietary P[23]. In plants, much of the P is in the form of phytate, which is poorly digested. Less P is therefore absorbed unless the food is supplemented with the enzyme phytase, e.g. by leavening bread with yeast producing phytase[24]. In addition, P in the form of phosphate additives in processed foods has been suggested to be almost completely absorbed[24].

Phosphate additives are commonly used in the food industry. The amounts used are generally below the recommended limits[25]; however, some products, e.g. low-fat processed cheeses, may contain nearly the maximum allowable amount of phosphate additives[26]. In Finland, other important sources of phosphate additives are confectioneries leavened with baking powder, plant extract drinks, sausages and other meat products, and most processed foods[26]. Nevertheless, accurate estimation of the intake of P from food additives is difficult, as the amounts of phosphate additives used in industrially prepared foods are not well known[26,27].

Earlier epidemiological studies have revealed unfavourable effects of phosphoric acid-containing soft drinks on bone[28–30]. However, few studies exist regarding the effects of other foods containing phosphate additives on Ca and bone metabolism, although soft drinks are not the main sources of phosphate additives at the population level. In the present cross-sectional study, our objective was to compare the effects of dietary P originating from natural P (NP) with that originating from phosphate additives (AP) on Ca and bone metabolism. We hypothesized that dietary P from AP would have a more deleterious impact on Ca and bone metabolism than dietary NP. Our earlier controlled short-term studies[12,13] established that high total dietary P intake has negative effects on Ca and bone metabolism. Our further objective was to investigate whether the same effects of total high dietary P intake could also be seen in the habitual diets of healthy individuals. We hypothesized that high total dietary P intake would have a more unfavourable impact than low intake on Ca and bone metabolism.

Participants and methods

Participants

Our data form part of a cross-sectional study conducted in southern Finland from February to March 1998. Our participants represent a randomly selected subgroup of 31- to 43-year-old Finnish women. Details about participants have been provided elsewhere[31]. 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. Power calculation based on S-PTH concentration (expected difference between groups in mean S-PTH concentration of 12 ng/l), assuming 90 % power with $\alpha = 0.05$, showed that a sample size of eleven in each group was adequate. In 1998, 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 information collection

A questionnaire was used to collect information on weight, height, physical activity, smoking habits and alcohol consumption of participants during the last two weeks in February or March 1998. In addition, the questionnaire requested information on age, age at menarche, past medical history, menstruation cycle, use of supplements and lactose intolerance.

Nutritional assessment

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 one day 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 (UNIDAP; Becel Palvelu Paasivaara Oy, Finland, 1989), based on the food composition database (Fineli) of the Finnish National Public Health Institute. Nutrient contents of all foods here are based on the food composition database (Fineli) of the Finnish National Public Health Institute[32].

Study design

To examine the effects of different sources of dietary P, we divided participants into groups based on the information provided in their 4 d food records. Group characteristics and their sizes are presented in Table 1. For total P intake we used quartiles. To test our hypothesis, we chose to investigate only the extreme total P quartiles and ignored the quartiles situated between them. This allowed us to study the total P intakes in a manner similar to our earlier controlled study designs[12,13]. Dairy products are the main P sources in the Finnish diet[3], with milk and cheese, excluding processed cheese, representing commonly consumed dairy products containing only NP. Due to skewed distribution of P in milk and cheese, participants were divided into two groups of equal size (low and high consumption) according to their median P intake. As processed cheese contains AP, it was

Table 1 Quartiles and groups studied

Quartiles and groups	P intake (mg/d)		No. of subjects
	Mean	SE	
Total P intake			
1st quartile	961	22	37
4th quartile	1956	48	35
Milk and cheese*			
Low consumption	244†	12	74
High consumption	588†	25	73
Processed cheese			
Non-consumers	0	–	110
Consumers	240‡	34	37

*Two groups of equal size (low and high consumption) according to the median intake of P from milk and cheese, excluding processed cheese (374 mg/d).
†P intake from milk and cheese, excluding processed cheese.
‡P intake from processed cheese.

examined separately from milk and other cheese than processed cheese. The small number of consumers of processed cheese forced us to use division into two groups of unequal size (consumers and non-consumers).

Sampling

On study days, blood samples were taken anaerobically between 07.30 and 09.15 hours after a 12 h overnight fast. The separated serum samples were stored at $-20°$C until analysed.

Laboratory measurements

The S-iCa concentration was analysed within 90 min of sample collection with an ion-selective analyser (Microlyte 6; Thermo Electron Corp., Vantaa, Finland). The intra-assay CV was 1·6 % for S-iCa. The serum intact PTH (S-iPTH) concentration was measured using an immunoradiometric assay (Nichols Institute, Juan San Capistrano, CA, USA) with 10–65 ng/l as a reference range. Intra- and inter-assay CV for S-iPTH were 3·7 % and 1·0 %, respectively. Serum 25-hydroxyvitamin D (S-25(OH)D) concentration was measured by RIA (Incstar Corp., Stillwater, MN, USA). The intra- and inter-assay CV were 10·1 % and 14·9 %, respectively.

Statistical analysis

Hypothesis and statistical approaches

Our data analysis was tailored to test the hypothesis 'Dietary AP affects bone metabolism more harmfully than dietary NP'. First, we estimated the effect of dietary AP by comparing means of S-PTH among consumers and non-consumers of processed cheese. Next, potential distortions of these averages were removed by adjusting them for the effects of relevant covariates. The choice of these covariates is discussed in the next paragraph. Finally, the two averages were adjusted for total P intake to exclude the effect of increased total P intake due to consumed processed cheese. The critical point with respect to testing our hypothesis is whether the difference between the

consumer groups prevails even after this adjustment; if so, our hypothesis gains support. With this same technique, we estimated the effect of dietary NP by comparing the averages of S-PTH among low and high milk and cheese (excluding processed cheese) consumption groups. The statistical approach originates from an elaboration technique[33], but we have used it in a more confirmatory spirit like e.g. Penttilä et al.[34] and Sah et al.[35]. We also tested the hypothesis 'High total dietary P intake affects bone metabolism more harmfully than low intake' by comparing averages of S-PTH in the first and fourth total P intake quartiles after adjusting for all relevant covariates. As S-iCa is a central factor in Ca and bone metabolism, we studied the effects of AP, NP and total P intake on S-iCa as well. Our technical tool for all data analysis was analysis of covariance (ANCOVA) because it enables inclusion of both categorical (e.g. use of contraceptives) and continuous (e.g. S-iCa) explanatory variables. We used the SPSS statistical software package version 12·07 (SPSS Inc., Chicago, IL, USA, 2003) in a Windows environment for all statistical analyses. Results were considered statistically significant at $P < 0·05$. Data are presented as means with their standard errors.

Covariates

Several factors are known to affect S-PTH concentration, which we use as a measure of Ca and bone metabolism. Among these are nutrient intakes (Ca, P and Na) and serum variables (S-25(OH)D and S-iCa). The use of contraceptives might also affect S-PTH[36]. When the means of S-iCa were compared, S-PTH was included in and Na intake excluded from covariates. We excluded age from the covariates, as all participants represented the same age group and all were premenopausal women. Energy intake was also excluded from the covariates because Ca and P intakes correlated well with energy intake. Furthermore, body weight had no effects on the variables measured, and thus was not included in the covariates. In summary, we chose covariates that correlated with the outcome parameter or covariates known to affect the outcome according to the literature.

Results

Baseline characteristics

The baseline characteristics of the 147 participants are presented in Table 2. Of all participants, thirty-nine (27 %) used contraceptives. The average dietary intake of P and Ca of participants (Table 2) corresponds to the average intake of P and Ca in Finnish females[3].

Effect of total dietary P intake on serum parathyroid hormone

The mean S-PTH concentration of participants was 30·6 (SE 1·3) ng/l. S-PTH concentration was above the upper reference limit (>65 ng/l) in two participants and below

Table 2 Basic characteristics of the study participants: healthy, premenopausal, Finnish women aged 31–43 years (*n* 147)
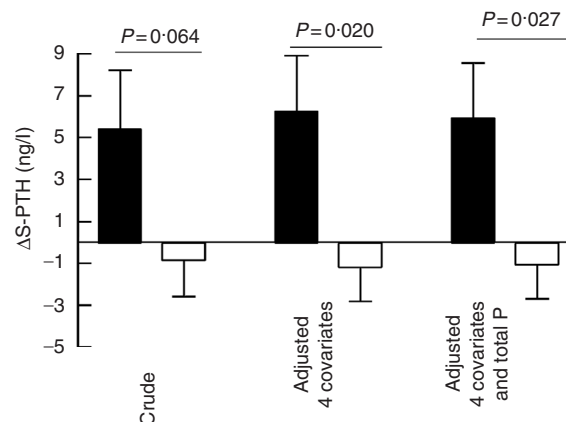
Variable	Mean	SE
Age (years)	38	0·27
Weight (kg)	64	0·85
Height (cm)	166	0·57
BMI (kg/m^2)	23	0·28
Habitual dietary energy intake (MJ/d)	7·9	0·19
Habitual dietary P intake (mg/d)	1411	32·7
Habitual dietary Ca intake (mg/d)	1056	33·6
Habitual dietary Ca:P ratio (mg:mg)	0·74	0·01
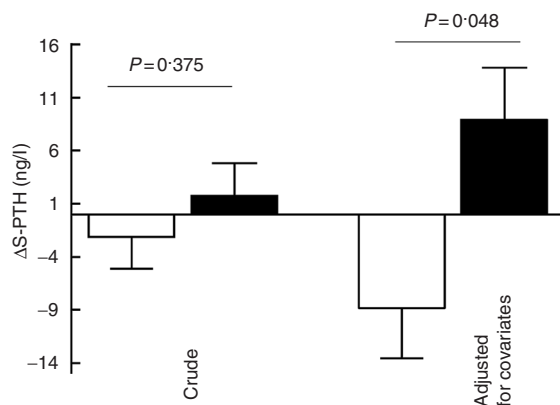
Fig. 1 Effects of total dietary phosphorus on serum parathyroid hormone (S-PTH) concentration in the first (□, *n* 37) and fourth (■, *n* 35) quartiles of total P intake among healthy, premenopausal, Finnish women aged 31–43 years. Values are means with their standard errors represented by vertical bars. Changes in S-PTH (ΔS-PTH) values were calculated from the mean S-PTH concentration (30·6 ng/l). The original mean S-PTH values adjusted for all covariates are presented in the Results section. The covariates used in the model included serum 25-hydroxyvitamin D and serum ionized calcium concentrations, total dietary calcium and sodium intakes, and use of contraceptives. Analysis of covariance was performed
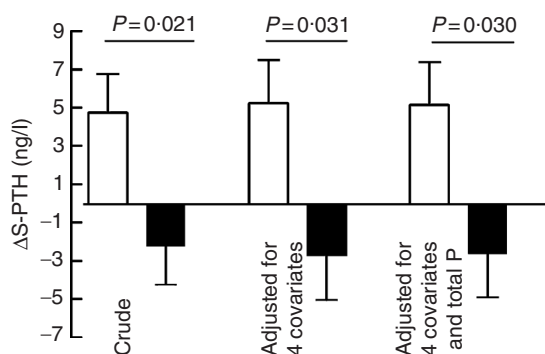

Fig. 2 Effects of processed cheese consumption on serum parathyroid hormone (S-PTH) concentration in the subgroups of consumers (■, *n* 30) and non-consumers (□, *n* 78) of processed cheese, who were not using contraceptives, among healthy, premenopausal, Finnish women aged 31–43 years. Values are means with their standard errors represented by vertical bars. Changes in S-PTH (ΔS-PTH) values were calculated from the mean S-PTH concentration (30·6 ng/l). The original mean S-PTH values adjusted for all covariates and total dietary phosphorus intake are presented in the Results section. The four covariates used in the model included serum 25-hydroxyvitamin D and serum ionized calcium concentrations, total dietary calcium and sodium intakes. At the final stage, total dietary phosphorus intake was added to the model. Analysis of covariance was performed

the lower reference limit (<10 ng/l) in two participants. The mean total P intake was 961 (SE 21·9) mg/d in the lowest P quartile (first quartile) and 1956 (SE 47·8) mg/d in the highest quartile (fourth quartile). Relative to the lowest total dietary P quartile, the mean S-PTH concentration was 1·8-fold higher in the highest quartile (S-PTH (ng/l): 21·7 and 39·4 for the lowest and the highest quartile, respectively; *P* = 0·048, ANCOVA) after adjustments for relevant covariates (Fig. 1).

Effect of phosphate additives on serum parathyroid hormone

The mean intake of P from AP-containing processed cheese among the processed cheese consumers was 240 (SE 34) mg/d. Compared with non-consumers (*n* 110), the mean S-PTH tended to be higher among consumers (*n* 37) of processed cheese (S-PTH (ng/l): 29·6 and 33·3 for non-consumers and consumers, respectively). However, the difference was not significant (*P* = 0·190, ANCOVA)

probably due to different group sizes and the significant interaction (*P* = 0·023, ANCOVA) found between processed cheese consumption and use of contraceptives. Due to this interaction, we studied the effects of AP-containing processed cheese on S-PTH in the subgroups of contraceptive users (*n* 39) and non-users (*n* 108). Among contraceptive non-users, we found that processed cheese consumption was associated with a higher mean S-PTH concentration than non-consumption (S-PTH (ng/l): 36·5 and 29·5 for consumers and non-consumers, respectively) after adjustment for covariates and total P intake (*P* = 0·027, ANCOVA; Fig. 2). Among contraceptive users, mean S-PTH did not differ significantly between consumers and non-consumers of processed cheese after adjusting for four covariates and total P intake (*P* = 0·153, ANCOVA).

Effect of natural P on serum parathyroid hormone

The mean P intake (*P* < 0·001, ANOVA) as well as the mean Ca intake (*P* = 0·009, ANOVA) from milk and cheese in the higher milk and cheese consumption group (*n* 73) was 2·4-fold higher than in the lower milk and cheese consumption group (*n* 74). The mean S-PTH concentration was greater with lower than with higher milk and cheese consumption (S-PTH (ng/l): 33·2 and 27·8 for lower and higher consumption, respectively; *P* = 0·065, ANCOVA) after adjustment for covariates and

Fig. 3 Effects of milk and cheese, excluding processed cheese, consumption on serum parathyroid hormone (S-PTH) concentration in the subgroups of low (□, n 59) and high (■, n 55) milk and cheese consumption, when serum ionized calcium concentration was <1·225 mmol/l, among healthy, premenopausal, Finnish women aged 31–43 years. Values are means with their standard errors represented by vertical bars. Changes in S-PTH (ΔS-PTH) values were calculated from the mean S-PTH concentration (30·6 ng/l). The original mean S-PTH values adjusted for all covariates and total dietary phosphorus intake are presented in the Results section. The four covariates used in the model included serum 25-hydroxyvitamin D, total dietary calcium and sodium intakes, and use of contraceptives. At the final stage, total dietary phosphorus intake was added to the model. Analysis of covariance was performed

total P intake. As a significant interaction between S-iCa concentration and consumption of milk and cheese (P = 0·009, ANCOVA) was found, we examined the effects of milk and cheese on S-PTH concentration in the subgroups of S-iCa < 1·225 mmol/l (n 114) and S-iCa ≥ 1·225 mmol/l (n 33). S-iCa = 1·225 mmol/l was the point at which the curves for the milk and cheese consumption groups intersected. With lower milk and cheese consumption, when S-iCa concentration was below 1.225 mmol/l, the mean S-PTH was greater than with higher consumption (S-PTH (ng/l): 35·7 and 28·0 for lower and higher consumption, respectively) even after adjustment for covariates and total P intake (P = 0·030, ANCOVA; Fig. 3). By contrast, with S-iCa concentration ≥1·225 mmol/l, the mean S-PTH concentrations between lower and higher milk and cheese consumption groups did not differ significantly (P = 0·364, ANCOVA) after adjustment for the four covariates and total P intake.

Effects of total dietary P, phosphate additives and natural P on serum ionized Ca

As shown in Table 3, total dietary P intake significantly affected S-iCa concentration. Compared with the lowest total dietary P quartile (n 38), the mean S-iCa concentration was lower in the highest P quartile (n 35; P = 0·016, ANCOVA) after adjustment for all relevant covariates. However, the mean S-iCa concentration did not differ between low and high milk and cheese consumption groups (P = 0·7, ANCOVA) or between consumers and non-consumers of processed cheese (P = 0·9, ANCOVA) after adjustment for all covariates (Table 3).

Table 3 Mean serum ionized calcium (S-iCa) concentration according to quartiles and groups of the studied participants: healthy, premenopausal, Finnish women aged 31–43 years (n 147)

Quartiles and groups	S-iCa (mmol/l)		No. of subjects
	Mean	SE	
Total P intake			
1st quartile	1·218†	0·007	37
4th quartile	1·188	0·008	35
Milk and cheese*			
Low consumption	1·204‡	0·003	74
High consumption	1·199	0·004	73
Processed cheese			
Non-consumers	1·202‡	0·003	110
Consumers	1·201	0·005	37

*Two groups of equal size (low and high consumption) according to the median intake of P from milk and cheese, excluding processed cheese (374 mg/d).
†Mean value was significantly different (P < 0·05) from that of the fourth quartile (analysis of covariance), after adjusting for total dietary Ca intake, serum parathyroid hormone (S-PTH) and serum 25-hydroxyvitamin D (S-25(OH)D) concentrations, and the use of contraceptives.
‡Mean value was not significantly different (P > 0·05) from that of the high consumption/consumers group (analysis of covariance), after adjusting for total dietary Ca and P intakes, S-PTH and S-25(OH)D concentrations, and the use of contraceptives.

Discussion

We observed unfavourable effects of high total dietary P intake on markers of Ca metabolism. Higher total habitual dietary P intake was associated with higher S-PTH and lower S-iCa concentrations even after total dietary Ca intake was equalized. Furthermore, we found that in the habitual diets of healthy individuals, foods containing AP had more harmful effects on Ca metabolism than foods containing NP. These effects were seen as higher S-PTH concentrations among those who consumed AP-containing foods. This difference may be due to the different bioavailability of P from AP and NP sources.

In line with the observations in our controlled short-term studies[12,13], we confirmed in the present cross-sectional study that habitual total dietary P affects Ca metabolism. As dietary P can affect S-PTH directly[17] or through S-iCa[16], we investigated the effects of dietary P intake on both variables. We found that the mean S-PTH was almost twofold higher and the mean S-iCa lower among participants whose habitual total P intake was the highest compared with those whose intake was the lowest. These findings are in accord with earlier intervention studies[10,11]. In fact, Calvo et al.[10] reported S-iCa concentrations to decrease in response to higher P (1660 mg/d) intake only in female participants not in males. Furthermore, in a 4-week intervention study in young men[37], P supplementation did not affect S-PTH, likely because of high dietary Ca intake, thus supporting the vital role of adequate Ca intake simultaneously with high dietary P intake. On the other hand, it has been found in earlier studies that effects of P have been greater in women than men[10] and in older than younger women[38]. However, the findings here suggest that habitually higher P intake has a more negative impact

than lower P intake on Ca and bone metabolism. The effect of total Ca intake was ruled out, as the effects of higher total P intake on S-PTH and S-iCa were observable even after total dietary Ca intake was equalized.

Processed cheeses contain both forms of P (NP and AP). The lower the fat content in processed cheese, the higher the P content[32] originating from AP[26]. When P intake is 775–1860 mg/d, 60–80 % of dietary P is absorbed in the intestine[22]. As AP-derived P is almost completely absorbed, the intake of P from such a source represents a larger burden in the human body. In the present study, we found that consumers of processed cheese had higher S-PTH concentrations than non-consumers. Our findings might reflect the negative influence of AP on Ca and bone metabolism found also in earlier intervention[10,11] and short-term[21] studies. In intervention studies[10,11], diets assembled from common foods, including processed foods with AP, increased S-PTH concentration in young adults. AP-containing foods included processed cheese, instant pudding and cola beverages. In postmenopausal women, cola beverage consumption resulted in higher S-PTH concentration and hypocalcaemia[28]. In cola beverages, phosphate additives are present in the form of phosphoric acid (H_3PO_4), while in other foods different forms of phosphate salts, e.g. Na polyphosphates, are used[26]. In our study, the intake of P from processed cheese was not high when compared with total dietary P intake. Therefore, these new findings suggest that P intake, which causes an increase in S-PTH, need not necessarily be high if P is derived from AP.

While P from AP increased S-PTH, the effects of P from NP from dairy products on S-PTH were contradictory. Milk and cheese (excluding processed cheese) are free of AP, but high in NP. In the present study, those who consumed more milk and cheese had lower mean S-PTH concentrations than those who consumed less. However, this difference did not exist when S-iCa concentration was at least 1·225 mmol/l. The high Ca content of milk and cheese probably explains the effect of higher consumption of these products on S-PTH. High Ca intake hinders the absorption of P in the intestine[23], and Ca supplementation has been found to suppress higher S-PTH concentrations induced by high P intake in healthy females[39]. Earlier findings support our results, as in postmenopausal women S-PTH decreased with increasing habitual dietary Ca intake despite simultaneously increasing habitual dietary P intake[40]. In addition, in healthy young females, consumption of fermented cheese in a short-term controlled diet decreased S-PTH concentrations and even decreased bone resorption[21]. The importance of a sufficient dietary Ca:P ratio in bone health is supported by the results of epidemiological[41–43], controlled[39] and intervention studies[11] in man as well as in animals[27,44]. However, other factors such as milk protein intake[45] or healthy eating habits may be linked to higher milk and cheese consumption, which might cause the favourable effect observed.

In conclusion, higher habitual total dietary P intakes were associated with higher mean S-PTH and lower mean S-iCa concentrations. We observed in the habitual diets of a randomly selected subgroup of women that AP might affect bone more negatively than other P sources, as indicated by higher mean S-PTH concentrations among participants consuming AP-containing foods. The effects of NP from milk and cheese, excluding processed cheese, on S-PTH were the opposite of those of AP-containing foods, probably due to higher Ca content in these foods. This may be important new information as the consumption of processed foods has increased during the last decades, which in turn has increased P intake from AP. The intakes of AP and total P have been shown to rise due to increasing consumption of fast, snack and convenience foods. High dietary P intake may no longer be a problem only in patients with impaired renal function, affecting also healthy individuals whose diet contains excessive P derived from AP. As the foods we examined represent only one type of NP and AP sources, the effects of other foods, e.g. meat or baked products, might be different because dairy products are the only foodstuffs containing large amounts of both P and Ca. Intervention studies with a wider selection of AP- and NP-containing foods are needed to confirm the present findings in healthy human subjects.

Acknowledgements

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