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Effects of dietary phosphorus and calcium-to-phosphorus ratio on calcium and bone metabolism in healthy 20- to 43-year-old Finnish women

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ACADEMIC DISSERTATION

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To Ripe

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Tiivistelmä, Finnish summary

Fosfori ja kalsium ovat molemmat luun perusrakennusaineita, joita tarvitaan kestävän luuston muodostumiseen ja ylläpitoon läpi elämän. Riittävän kalsiumin saannin merkityksestä luuston hyvinvoinnille on vahvaa tutkimusnäyttöä, mutta fosforiin liittyviä tutkimuksia on tehty vain muutamia terveillä ihmisillä. Nämä aiemmat tutkimukset ovat kuitenkin antaneet viitteitä siitä, että runsas fosforin saanti, etenkin yhdistettynä vähäiseen kalsiumin saantiin, olisi haitallista luustolle kohonneen lisäkilpirauhashormonipitoisuuden välityksellä terveillä henkilöillä. Samanaikaisesti, kun kalsiumin saanti ravinnosta monilla länsimaalaisilla jää liian vähäiseksi, saadaan fosforia ravinnosta 2-3-kertaisesti yli ravitsemussuositusten. Tässä väitöskirjassa tutkittiin, onko ravinnon fosforimäärällä ja -lähteillä vaikutusta kalsiumin ja luun aineenvaihduntaan. Lisäksi tutkittiin ruokavalion kalsiumin ja fosforin saantimäärien, ja -suhteiden vaikutusta kalsiumin ja luun aineenvaihdunnan keskeisiin merkkiaineisiin. Väitöskirjassa keskityttiin tutkimaan terveitä suomalaisia 20–43-vuotiaita naisia.

Väitöskirjatyön ensimmäisessä kokeellisessa tutkimuksessa 20–28-vuotiailla naisilla (n=14) tutkittiin erisuuruisten fosforiannosten vaikutuksia. Lisäksi poikkileikkaustutkimusasetelmassa tutkittiin 31–43-vuotiaiden naisten (n=147) ruokavalioistaan saamien fosforimäärien yhteyttä kalsiumin ja luun aineenvaihduntaan sekä sitä, onko lisäainefosfaatin ja luontaisen fosforin välillä eroa. Väitöskirjatyön toisessa kokeellisessa tutkimuksessa selvitettiin voidaanko kalsiumin saantia lisäämällä vähentää runsaan ravinnon fosforin saannin aikaan saamia vaikutuksia 20–40-vuotiailla naisilla (n=12). Lisäksi tutkittiin ravinnon kalsium-fosforisuhteen yhteyttä kalsiumin ja luun aineenvaihduntaan poikkileikkaustutkimuksessa 31–43-vuotiailla naisilla (n=147).

Kokeelliset tutkimukset olivat kontrolloituja ajan ja ravinnon suhteen, ja tutkimuspäivien järjestys oli satunnaistettu. Jokainen tutkittava toimi itse itsensä kontrollina. Kokeellisten tutkimusten tutkimuspäivien aikana tutkittavilta kerättiin vuorokausivirtsanäytteet ja otettiin 5-6 verinäytettä tutkimuksesta riippuen jokaisen 24-h tutkimusvuorokauden aikana. Ensimmäisessä kokeellisessa tutkimuksessa tutkittavat saivat kolmena tutkimuspäivänä aterioiden yhteydessä fosforilisää, josta saatava fosforiannos (250 mg, 750 mg ja 1500 mg) vaihteli tutkimuspäivinä. Yksi tutkimuspäivä oli kontrollipäivä, jolloin fosforilisää ei nautittu, vaan aterioista saatava fosfori (495 mg/vrk) oli ainoa fosforinlähde. Tutkimuspäivän aterioista tutkittavat saivat kalsiumia 250 mg/vrk. Toisessa kokeellisessa tutkimuksessa tutkittavien ruokavaliosta saama fosforimäärä oli runsas (1850 mg/vrk) vastaten määrältään ensimmäisen kokeellisen tutkimuksen suurimman fosforiannoksen fosforin päiväsaantia. Tutkimuksessa tutkittavat saivat aterioiden yhteydessä kahtena tutkimuspäivänä kalsiumlisää, josta saatava kalsiumannos (600 mg ja 1200 mg) vaihteli. Kontrollipäivänä kalsiumlisää ei nautittu, jolloin ravinnosta saatava kalsium (480 mg/vrk) oli ainoa päivän kalsiumlähde. Poikkileikkaustutkimuksessa tutkittavat pitivät neljän vuorokauden ajan ruokapäiväkirjaa, ja tutkimuksen aikana heiltä kerättiin paastoverinäytteitä ja kolme kertaa vuorokausivirtsanäytteet. Ravintoaineiden saanti laskettiin ravintolaskentaohjelmalla ruokapäiväkirjanpitotiedoista. Sekä kokeellisissa että

poikkileikkaustutkimuksissa kerätyistä näytteistä määritettiin keskeisten kalsium ja luun aineenvaihduntaa kuvaavien merkkiaineiden pitoisuudet.

Ensimmäisessä kokeellisessa tutkimuksessa ravinnon fosforimäärä annosvastemaisesti kohotti seerumin fosfaatti- (S-Pi) ja lisäkilpirauhashormonipitoisuutta (S-PTH). Lisäksi suurin fosforiannos (1500 mg/vrk) laski seerumin ionisoituneen kalsiumin (S-iCa) pitoisuutta ja vähensi luun muodostusta (S-BALP) ja lisäsi hajoamista (U-NTx) sekä vaikutti kaikkiin muihinkin merkkiaineisiin haitallisimmin kaikista tutkimuksessa käytetyistä fosforiannoksista. Toisessa kokeellisessa tutkimuksessa ravinnon fosforin saannin ollessa runsasta kalsiumin lisääminen ruokavalioon annosvastemaisesti laski S-PTH pitoisuutta ja U-NTx eritystä sekä kohotti S-iCa pitoisuutta ja täten vaikutti edullisesti kalsiumin ja luun aineenvaihduntaan. Silti kalsiumannoksilla ei havaittu olevan vaikutusta luun muodostukseen, kun ravinnon fosforimäärä oli suuri, mikä viittaa siihen, että tutkimuksessa käytetyillä kalsiumannoksilla (1080 ja 1680 mg/vrk) ei pystytty vähentämään kaikkia runsaan fosforin saannin aiheuttamia haitallisia vaikutuksia. Liiallista kalsiumin saantia tulee kuitenkin välttää, sillä se voi aiheuttaa muita terveydellisiä ongelmia.

Poikkileikkaustutkimuksessa runsas ravinnon fosforin saanti oli yhteydessä kohonneisiin S-PTH ja matalampiin S-iCa pitoisuuksiin. Fosforilähteistä lisäainefosfaatteja sisältävän ruoan käyttö oli yhteydessä korkeampiin S-PTH pitoisuuksiin. Sen sijaan luontaisen fosforin lähteiden runsaampi kulutus oli yhteydessä matalampiin S-PTH pitoisuuksiin kuin vähäisempi kulutus. Poikkileikkaustutkimuksessa havaittiin myös, että alhainen ravinnon kalsium-fosforisuhde ($\leq$0.50, moolisuhde) oli yhteydessä samanaikaisesti kohonneisiin S-PTH pitoisuuksiin ja runsaampaan virtsan kalsium erityksen (U-Ca), mikä viittaa siihen, että alhainen ravinnon kalsium-fosforisuhde voi häiritä kalsiumaineenvaihduntaa ja lisätä luun hajoamista. Lisäksi, vaikka tutkittavien kalsiumin saanti oli riittävää tai runsasta, ei kukaan tutkittavista saavuttanut ruokavaliossaan suositeltavaa kalsium-fosforimooli-suhdetta 1. Tämä johtui tutkittavien 2-3-kertaa ravitsemussuositukset (600 mg/vrk) ylittävästä fosforin saannista.

Yhteenvetona tutkimuksista todetaan, että terveiden suomalaisnaisten runsas fosforin saanti näyttää olevan haitallista kalsiumin ja luun aineenvaihdunnalle, varsinkin, jos kalsiumin saanti ravinnosta on vähäistä. Runsaan fosforin saannin haitalliset vaikutukset havaittiin sekä kokeellisissa että poikkileikkaustutkimuksissa. Lisäksi kokeellisessa tutkimuksessa runsas fosforin saanti lisäsi luun hajoamista ja vähensi muodostusta, mikä on haitallista luuston hyvinvoinnille pitkällä aikavälillä. Tämän väitöskirjatyön tutkimustulokset viittaavat myös siihen, että lisäaineista peräisin oleva fosfori on haitallisempaa kuin elintarvikkeen luontaisesti sisältämä fosfori. Vaikka tämän väitöskirjatyön tutkimukset osoittivat, että ravinnon runsaan fosforin saannin haitallisia vaikutuksia voidaan vähentää, niin silti niitä ei voida kokonaan poistaa ruokavaliolla, joka sisältää riittävästi kalsiumia. Runsaan fosforin saannin vähentäminen näyttäisi olevan tämän väitöskirjatyön tulosten perusteella perustelua myös terveillä ihmisillä.

Abstract

Phosphorus (P) and calcium (Ca) are essential minerals for bone and are needed for optimal bone health throughout life. The importance of adequate Ca intake for the skeleton is well established. Less is known, however, about the role of dietary P in bone health, especially in healthy individuals. Some earlier studies have suggested that an excessively high dietary P intake could be deleterious to bone through increased parathyroid hormone (PTH) secretion, but the effects of excessive P intake are poorly understood in healthy humans. While the intake of Ca in many Western countries remains below recommended levels, the intake of P exceeds 2- to 3-fold the dietary guidelines. In this thesis, the effects of different dietary P intakes and sources on Ca and bone metabolism were investigated. As the metabolism of Ca and P is tightly bound together, the combined effects of Ca and P intakes on the central markers of Ca and bone metabolism were also determined. Healthy 20- to 43-year-old Finnish women were studied.

In the first controlled study with 20- to 28-year-old women (n = 14), we examined the effects of P doses, and in a cross-sectional study with 31- to 43-year-old women (n = 147) the associations of habitual P intakes with Ca and bone metabolism. In this same cross-sectional study, we also investigated whether differences exist between dietary P originating from natural P and phosphate additives. The second controlled study investigated whether by increasing the Ca intake, the effects of a high P intake could be reduced in 20- to 40-year-old women (n = 12). The second controlled study was a sequel to the first one. In addition, the associations of habitual dietary calcium-to-phosphorus ratios (Ca:P ratio) with Ca and bone metabolism were determined in a cross-sectional study design with 31- to 43-year-old females (n = 147).

In controlled studies, the order of the study days was randomized, and within these studies all study day meals were identical for each subject on each study day. Each participant served as her own control. In both controlled studies, 24-h urine collections were performed and 5-6 blood samples were taken in each 24-h study session. In the first controlled study with four study days, the participants were given, in a randomized session order, 0 (placebo), 250, 750 or 1500 mg of P during the study day meals. In a placebo session, no additional P was given; meals were therefore the only sources of P, providing 495 mg/d of P and 250 mg of Ca. In the second controlled study with three study days, the participants had a high P intake (1850 mg/d) derived from study day meals. The P intake corresponded with the highest P dose in the first controlled study. Participants were given, in a randomized session order 0 (placebo), 600 or 1200 mg of Ca during the study day meals. In the placebo session, no additional Ca was given; thus, meals were the only source of Ca, providing 480 mg/d of Ca. In the cross-sectional studies, participants kept a 4-day food record, and during the study, fasting blood samples and three separate 24-h urinary samples were collected. The habitual dietary intake of the participants based on the 4-day food records was calculated with computer-based programs. In all studies, the central Ca and bone metabolism markers in blood and urine samples were measured by laboratory analyses.

In the first controlled study, the P dose dependently increased serum phosphate (S-Pi) and serum PTH (S-PTH) concentrations. In addition, the highest P dose (1500 mg/d) decreased serum ionized calcium (S-iCa) concentration and bone formation (S-BALP) and increased bone resorption (U-NTx). Thus, the highest P dose had the most negative effects on all markers measured. In the second controlled study, when P intake was high, increasing Ca intakes elevated S-iCa concentration and decreased S-PTH concentration and bone resorption (U-NTx) in a dose-dependent manner, thus having beneficial effects on Ca and bone metabolism. However, not even a high Ca intake could affect bone formation (S-BALP) when dietary P intake was excessive. This suggests that higher doses of Ca than those used in this study are needed to prevent the effect of excessive P intake. However, high Ca doses may increase the incidence of other serious diseases.

In the cross-sectional studies, a higher habitual dietary P intake was associated with lower S-iCa and higher S-PTH concentrations. In addition, the consumption of phosphate additive-containing foods was associated with a higher S-PTH concentration, while a higher consumption of natural P sources was associated with lower S-PTH concentrations than a lower consumption of such products. Moreover, habitual low dietary Ca:P ratios ($\leq$0.50, molar ratio) were associated with higher S-PTH concentrations and 24-h U-Ca excretions, suggesting that low dietary Ca:P ratios may interfere with homeostasis of Ca metabolism and increase bone resorption. In addition, the Ca intake of participants was mostly adequate, but none of the participants achieved the suggested Ca:P molar ratio of 1 in their habitual diets. This was mostly due to the dietary P intake being 2- to 3-fold higher than the recommended levels (600 mg/d).

In summary, excessive dietary P intake in healthy Finnish women seems to be detrimental to Ca and bone metabolism, especially when dietary Ca intake is low. The effects of high P intake were observable in both cross-sectional and controlled studies. Moreover, according to the findings in the controlled study, high P intake increased bone resorption and decreased bone formation, which could harm bone health. In addition, these findings imply that phosphate additives may be more harmful than natural P. The results of both the controlled and the cross-sectional studies indicate that by increasing dietary Ca intake to the recommended level, the negative effects of high P intake could be diminished, but not totally prevented. Thus, reduction of an excessively high dietary P intake is also beneficial for healthy individuals.

List of original publications

This thesis is based on the following original publications, referred to in the text by their Roman numerals (I-IV):

I **Kemi VE**, Kärkkäinen MUM, Lamberg-Allardt CJE. High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. Br J Nutr 2006;96:545-552.

II **Kemi VE**, Kärkkäinen MUM, Karp HJ, Laitinen KAE, Lamberg-Allardt CJE. Increased calcium intake does not completely counteract the effects of increased phosphorus intake on bone: an acute dose-response study in healthy females. Br J Nutr 2008;99:832-839.

III **Kemi VE,** Rita HJ, Kärkkäinen MUM, Viljakainen HT, Laaksonen MM, Outila TA, Lamberg-Allardt CJE. Habitual high phosphorus intakes and foods with phosphate additives negatively affect serum parathyroid hormone concentration: a cross-sectional study on healthy premenopausal women. Public Health Nutr 2009;12:1885-1892.

IV **Kemi VE**, Kärkkäinen MUM, Rita HJ, Laaksonen MML, Outila TA, Lamberg-Allardt CJE. Low calcium:phosphorus ratio in habitual diets affects serum parathyroid hormone concentration and calcium metabolism in healthy women with adequate calcium intake. Br J Nutr 2010;103:561-568.

In addition, some unpublished results are presented.

Contribution of authors to papers I-IV

I The first author (VEK) planned the study with co-authors. VEK recruited study subjects, conducted the experimental work and did most of the laboratory analysis. The laboratory technician and co-author Merja Kärkkäinen (MUMK) also contributed to the laboratory analysis. VEK carried out statistical analysis and wrote the manuscript. The co-authors critically reviewed the paper.

II VEK planned the study with MUMK and Christel Lamberg-Allardt (CJEL-A). VEK recruited study subjects and conducted the experimental work. VEK conducted the laboratory analysis together with MUMK and Heini Karp (HJK). Kalevi Laitinen (KAEL) served as medical advisor. VEK carried out the statistical analysis and wrote the manuscript. The co-authors critically reviewed the paper.

III VEK planned this sub-study with Hannu Rita (HJR) and CJEL-A. CJEL-A, MUMK, Marika Laaksonen (MML) and Terhi Outila (TAO) conducted the experimental work and the laboratory analysis of the original study in 1998. VEK calculated participants' dietary intakes and conducted the data analysis for this sub-study. VEK carried out the statistical analysis with the guidance of HJR. Heli Viljakainen (HTV) and MML assisted VEK with the statistical analysis. VEK prepared the manuscript for publication, and the co-authors participated by assisting with manuscript revision.

IV VEK planned this sub-study with MUMK, HJR and CJEL-A. CJEL-A, MUMK, MML and TAO conducted the experimental work and the laboratory analysis of the original study in 1998. VEK calculated participants' dietary intakes, conducted the data analysis and carried out the statistical analysis for this sub-study. VEK prepared the manuscript for publication, and the co-authors critically reviewed this manuscript.

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
25(OH)D	25-hydroxyvitamin D, calcidiol
AI	adequate intake
ALP	alkaline phosphatase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP	adenosine triphosphate
BALP	bone-specific alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
Ca	calcium
CaBP	calcium binding protein
Ca:P ratio	calcium-to-phosphorus ratio
CTx	carboxy-terminal telopeptide of collagen type I
CV	coefficient of variation
DPD	deoxypyridinoline
EIA	enzyme immunoassay
ELISA	enzyme-linked immunoassay
FFQ	food frequency questionnaire
FGF-23	fibroblast growth factor 23
GTP	guanosine triphosphate
Hypro	hydroxyproline
iCa	ionized calcium
ICTP	carboxy-terminal telopeptide of collagen type I
iPTH	intact parathyroid hormone
IRMA	immunoradiometric assay
LOAEL	lowest-observed-adverse-effects level
LSD	Fisher's least significant difference *post hoc* test
Na	sodium
NPT	Na-phosphate-cotransporter
NTx	cross-linked N-telopeptides of type I collagen
OC	osteocalcin
P	phosphorus
P_2O_5	phosphorus pentoxide
PTH	parathyroid hormone
Pi	phosphate
PICP	carboxyterminal propeptide of type I collagen
PINP	aminoterminal propeptide of type I procollagen
PYD	pyridinoline
RDA	recommended dietary allowance
RIA	radioimmunoassay
S-$1,25(OH)_2D$	serum 1,25-dihydroxyvitamin D
S-25(OH)D	serum 25-hydroxyvitamin D
S-ALP	serum alkaline phosphatase

S-BALP	serum bone-specific alkaline phosphatase
S-Ca	serum calcium
S-Cr	serum creatinine
S-CTx	serum carboxy-terminal telopeptide of collagen type I
SD	standard deviation
S-DPD	serum deoxypyridinoline
SEM	standard error of mean
S-FGF-23	serum fibroblast growth factor 23
S-iCa	serum ionized calcium
S-OC	serum osteocalcin
S-Pi	serum phosphate
S-PICP	serum carboxy-terminal propeptide of type I procollagen
S-PINP	serum amino-terminal propeptide of type I procollagen
S-PTH	serum parathyroid hormone
S-PYD	serum pyridinoline
TRACP 5b	tartrate-resistant acid phosphatase 5b
U-Ca	urinary calcium excretion
U-Cr	urinary creatinine excretion
U-DPD	urinary deoxypyridinoline excretion
U-Hyp	urinary hydroxyproline excretion
U-Na	urinary sodium excretion
U-NTx	urinary cross-linked N-telopeptides of type I collagen excretion
U-Pi	urinary phosphate excretion
U-PYD	urinary pyridinoline excretion
VDR	vitamin D receptor

1 Introduction

Osteoporosis is considered a major public health problem in developed countries and a costly disease worldwide (e.g. Kannus *et al.* 1999, International Osteoporosis Foundation 2004, Johnell and Kanis 2006). In the ageing societies of Western countries, osteoporosis is becoming an increasingly severe disease, as osteoporotic fractures cause more disability than many other chronic diseases (Johnell and Kanis 2006). Although the number of osteoporotic fractures is mainly explained by the ageing of the population, unhealthy dietary habits and an inactive lifestyle also affect the incidence of fractures (Kannus *et al.* 1999, Cummings and Melton 2002).

Of the total bone mass, 60-80% is determined by genes (Nguyen *et al.* 1998, Hunter *et al.* 2001). However, lifestyle factors, such as physical activity (Welten *et al.* 1994) and nutrition (Robins and New 1997, Bonjour *et al.* 2009a), also play a role in determining bone mass. From a nutritional point of view, the importance of adequate vitamin D and calcium (Ca) intake for bone health is well established (Welten *et al.* 1995, Bischoff-Ferrari *et al.* 2005, Tang *et al.* 2007). Nevertheless, the role of dietary phosphorus (P) in bone health, especially in healthy individuals, is less clear. Some earlier intervention studies have suggested that an excessively high dietary P intake could be deleterious to bone through increased parathyroid hormone (PTH) secretion (Calvo *et al.* 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996), but the effects of excessive P intakes are poorly understood in healthy humans. The primary focus of this thesis was to investigate how P intakes commonly found in Western diets affect Ca and bone metabolism.

P is an essential nutrient for the skeleton and P deficiency causes rickets in children (Pettifor 2008) and osteomalacia in adults (Genant 1993). Nevertheless, P deficiency is seldom due to a low dietary intake of P, but rather to a genetic disorder. In fact, while dairy products are the main sources of Ca, P is readily available in a wide range of foodstuffs, as foods can contain both natural P and phosphate-containing food additives. Furthermore, dietary habits have changed during the past decades towards an increasing consumption of processed foods, which has notably increased not only total dietary P intake, but also intake of P from phosphate additives (Calvo 2000, Suurseppä *et al.* 2001). Therefore, another aim here was to compare the associations of dietary P originating from natural P and from phosphate additives with Ca and bone metabolism.

The usual daily P intake in a typical Western diet exceeds by 2- to 3-fold (Calvo 1993, Gronowska-Senger and Kotanska 2004, EFSA 2005) the recommended RDA for P intake (700 mg/d) (Food and Nutrition Board 1997). The same trend is also seen in Finland, as the latest results have revealed that the mean P intake of 25- to 64-year-old women is 1363 mg/d, and in the same age group of men 1778 mg/d (Paturi *et al*. 2008), while the recommended intake for P is 600 mg/d (National Nutrition Council 2005). Unlike in many other Western countries (e.g. Bryant *et al.* 1999, Guéguen and Pointillart 2000, Lombardi-Boccia *et al.* 2003), the dietary Ca intake in Finland is in general adequate or high. The

mean Ca intake of 25- to 64-year-old Finnish women is 1007 mg/d and that of men 1202 mg/d (Paturi *et al.* 2008), thus meeting the Finnish nutritional guidelines for Ca (800 mg/d) (National Nutrition Council 2005). However, if the habitual diet lacks dairy products, the dietary Ca intake and dietary calcium-to-phosphorus ratio (Ca:P ratio) easily drops far below the optimal level (Ca:P molar ratio of 1) (e.g. SCF 1993, Calvo and Park 1996). In fact, very low Ca:P ratios (≤0.25) have been reported in the diets of young girls and boys, teenagers and young adults (Calvo 1993, Chwojnowska *et al.* 2002). In several animal studies, diets low in Ca and high in P negatively affected bone health (for review see Calvo and Park 1996). Relative to the number and quality of animal studies, the effects of dietary Ca:P ratios on Ca and bone metabolism in humans have been infrequently investigated. Therefore, as the metabolism of Ca and P is tightly bound together, studying not only the effects of dietary P *per se,* but also the combined effects of Ca and P intakes on Ca and bone metabolism is essential.

While a high dietary P intake is known to have deleterious consequences for renal patients, as they have impaired ability to excrete P, could an excessive dietary P intake be a problem for healthy individuals, too? This is discussed in this work. Information on how dietary P is metabolized, different sources of P and the combined effects of dietary Ca and P on Ca and bone metabolism in healthy 20- to 43-year-old Finnish women is provided.

2 Review of the literature

2.1 Phosphorus and calcium

2.1.1 Dietary sources

2.1.1.1 Phosphorus sources

Phosphorus (P) is abundant in many food sources, as foods can contain both natural P and phosphate additives. Foods high in protein are also high in natural P. In Finland, the main dietary sources of P are dairy, grain and meat products (Paturi *et al.* 2008). The P content of foodstuffs varies between 0 and 1570 mg/100 g of product (National Institute for Health and Welfare 2009) (Table 1). In some countries, dietary supplements may also contain P as phosphates (EFSA 2005, Uribarri 2007). Protein bars and products used to build muscle mass may have high P content (EFSA 2005).

Table 1. *Phosphorus (P) content of selected foods.*

Food	mg P/100 g	Food	mg P/100 g
Dairy products		*Meat products*	
Cheese, average	522	Chicken with skin	130
Milk	90	Ham, boiled*	200
Processed cheese, 9-12 g fat*	1570	Minced meat, beef 17% fat	146
Processed cheese, 20-24 g fat*	610	Sausage, average*	108
Processed cheese, 27-35 g fat*	360	Turkey, cold cuts*	260
Yoghurt, berries and fruit	120	*Others*	
Grain products		Cola beverages*	15
Macaroni, boiled	42	Egg	210
Rye bread, 51% rye	212	Fish, average	252
Rice, boiled	45	Potato, boiled	45
White bread, made with water	78	Peas	130

* Contains phosphate additives
Source: National Food Data Base Fineli®, National Institute for Health and Welfare

Due to increased use of phosphate additives in the food industry and the rising consumption of processed foods, authors in Finland (Blomberg and Penttilä 1999, Suurseppä *et al.* 2001) and USA (e.g. Uribarri and Calvo 2003, Uribarri 2009) have reported that the intake of P from phosphate additives has increased in the past decades. In Finland, the use of phosphate additives is regulated by the decision of the Ministry of Trade and Industry (No. 811/1999), in which the use of phosphate additives is restricted

by set maximum amounts and prohibition of their use in certain foods (Ministry of Trade and Industry 1999). Consumers can recognize phosphate additives as E-codes on food labels (Table 2) (Finnish Food Safety Authority 2009).

Table 2. *E-codes of the most commonly used phosphate additives.*

Phosphate additive	E-code	Phosphate additive	E-code
Phosphoric acid	E338	Disodium diphosphate Trisodium diphosphate Tetrasodium diphosphate	E450
Monosodium phosphate Disodium phosphate Trisodium phosphate	E339	Dipotassium diphosphate Tetrapotassium diphosphate Dicalcium diphosphate Monocalcium diphosphate	E450
Monopotassium phosphate Dipotassium phosphate Tripotassium phosphate	E340	Pentasodium triphosphate Pentapotassium triphosphate	E451
Monocalcium phosphate Dicalcium phosphate Tricalcium phosphate	E341	Sodium polyphosphate Potassium polyphosphate Sodium calcium polyphosphate Calcium polyphosphate	E452
Monomagnesium phosphate Dimagnesium phosphate	E343	Sodium aluminium phosphate, sour	E541

Source: Elintarvikkeiden lisäaineiden E-koodiavain, Finnish Food Safety Authority

Phosphate additives, which are either phosphoric acid or varied phosphate salts, are used to sequester metal ions, to act as buffers, to increase water binding, to adjust pH, to serve as an anti-caking agent, to form ionic bridges, to interact with proteins and other charged hydrocolloids and to prevent loss of carbonation caused by heavy metals and acidifications in beverages (Suurseppä *et al.* 2001, Murphy-Gutekunst 2005, Murphy-Gutekunst and Uribarri 2005, Karalis and Murphy-Gutekunst 2006). When Finland joined the European Union in 1995, the maximum allowable amounts of phosphate additives increased due to EU legislation. This elevated the use of phosphate additives in the food industry. The use of phosphate additives in certain foods even doubled with the tripling of the permitted level compared with the previous level in Finland before 1995 (Blomberg and Penttilä 1999). However, the amounts used are generally below the permitted limits (Blomberg and Penttilä 1999), although some foods, e.g. low-fat processed cheeses, may contain nearly the maximum allowable amount of phosphate additives (Suurseppä *et al.* 2001). In Finland, other important sources of phosphate additives are confectioneries leavened with baking powder, plant extract drinks (e.g. cola beverages), sausages and other meat products and most processed foods (Suurseppä *et al.* 2001). Due to the high consumption rate of sausages and other meat products, processed foods and bakery products, these foods are important sources of phosphate additives in Finland.

2.1.1.2 Calcium sources

For adults in Finland, dairy products are the main sources of calcium (Ca), as ≥62% of Ca intake is derived from milk or other dairy products (Paturi *et al.* 2008). Of all foods, dairy products have the highest Ca content (National Institute for Health and Welfare 2009). The amount of Ca is similar in low- and high-fat dairy products. Fish are also good source of Ca if they are consumed with bones. Ca content in plants is notable only in seeds, almonds and peanuts. In addition, kale, spinach, beans, cabbage and oranges contain Ca (Table 3). Ca content in foods varies between 0 and 1050 mg/100 g of product (National Institute for Health and Welfare 2009) (Table 3).

Nowadays, there are still some groups of people, e.g. those who are lactose intolerant, allergic to milk or vegan, who do not consume or seldom consume dairy products. In addition, in pregnancy or during lactation, Ca intake needs to be increased (National Nutrition Council 2005). Therefore, Ca supplements or Ca-fortified foods are an option for these individuals. Food manufacturers have developed Ca-fortified foods, and Ca has been added to, for example, mineral water and juices (Raulio and Suojanen 2000, Hirvonen *et al.* 2004). In France, mineral water is considered a good source of Ca (Guillemant *et al.* 2000). However, Ca content of mineral waters differs between and within countries. In Finland, Ca supplements are widely used among women; 23.7% of women, but only 6.6% of men reported using Ca supplements in 2007 (Paturi *et al.* 2008).

Table 3. *Calcium content of selected foods.*

Food	mg Ca/100 g	Food	mg Ca/100 g
Dairy products		*Others*	
Cheese, Emmental, 27-30 g fat	939	Fish, average, fried	118
Curd	117	Kale	42
Ice cream, cream-based	146	Orange, without skin	54
Milk	120	Peanut	78
Processed cheese, 9-12 g fat	600	Sesame seeds, with hull	975
Yoghurt, 1.5% fat	150	Spinach	88

Source: National Food Data Base Fineli®, National Institute for Health and Welfare

2.1.1.3 Calcium-to-phosphorus ratio of foods

Based on the calculations of the recommended dietary Ca and P intakes, the optimal dietary calcium-to-phosphorus molar ratio (Ca:P molar ratio) is suggested to be 1 (SCF 1993, Calvo and Park 1996, National Nutrition Council 2005), corresponding to a Ca:P weight ratio of 1.3. Weight Ca:P ratios (mg:mg) can be converted into molar ratios (mol:mol) by using molecular weight of Ca (40.08 g/mol) and P (30.97 g/mol). Ca:P ratios in foods vary, being highest in dairy products (Table 4). By adding phosphate additives to the foods, the Ca:P ratio drops. In the EU, infant formula and follow-on formula should

have a Ca:P weight ratio between 1.2 and 2.0 (EFSA 2005). Concerning foods in adult diets, only dairy products have Ca:P ratios equal or close to the suggested dietary Ca:P weight ratio of 1.3 (Table 4). The Ca:P ratio in bread or meat products will increase when milk or milk powder is used as an ingredient.

Table 4. *Calcium-to-phosphorus weight ratio (Ca:P ratio) of selected foods.*

Food	Ca:P ratio (mg:mg)	Food	Ca:P ratio (mg:mg)
Dairy products		*Meat products*	
Cheese, average	1.55	Chicken (boiled)	0.07
Processed cheese, 9-12 g fat*	0.38	Ham, boiled*	0.03
Processed cheese, 20-24 g fat*	0.96	Sausage, average*	0.16
Processed cheese, 27-35 g fat*	1.18	Sausage, dry, salami type	0.08
Milk, average	1.35	Turkey, cold cuts*	0.02
Grain products		*Others*	
Rye bread, 51% rye	0.09	Egg (boiled)	0.27
White bread, made with water	0.17	Fish (fried) average	0.39
White bread, made with milk	0.51	Cola beverages*	0.20

* Contains phosphate additives
Source of original Ca and P content of foods, in which calculated Ca:P ratios are based on the National Food Data Base Fineli® provided by the National Institute for Health and Welfare

2.1.2 Dietary guidelines and dietary intakes

2.1.2.1 Phosphorus

In Finland, the recommended intake of P for adults is 600 mg/d (National Nutrition Council 2005). For individuals aged 18-20 years, the intake is suggested to be slightly higher (700 mg/d), corresponding to the recommended RDA of P intake for adults in the United States (Food and Nutrition Board 1997). Recommendations made by the Food and Nutrition Board (1997) are based on the maintenance of serum phosphate concentration (S-Pi) within the normal adult range. The recommendations for younger age groups, take into account P accretion in bone and lean tissues. In 1993, the Scientific Committee for Foods in Europe suggested that for adults the average daily requirement for P is 400 mg, the population reference intake 550 mg and the lowest threshold intake 300 mg (SCF 1993). In the Finnish Nutrition Recommendations, 5 g/d is set as the lowest limit for adverse effects of P, whereas the Institute of Medicine (1997) has set an upper reference limit (URL) of 4 g/d for P intake in adults (Food and Nutrition Board 1997). While the European Food Safety Authority (EFSA) has not established an upper limit for P intake (EFSA 2005), they concluded in their report that “based on available knowledge, normal healthy individuals can tolerate P intakes of up to 3 g/d”. Nevertheless, some individuals

might be more vulnerable to high P intakes, with deleterious effects seen as gastrointestinal symptoms. In 1975, the Life Science Research Office (LSRO) in the USA evaluated the risks of phosphate additives on the health of American consumers, and they listed several phosphate additives to be safe for use in food processing at the levels, used in the 1970s (LSRO 1975). Since then, the consumption of processed foods has increased significantly, which has increased the intake of P from phosphate additives, but the recommendations made in 1975 have not been updated.

In the FINDIET 2007 survey, the mean dietary intake of P among 25- to 64-year-old Finns was 1326 mg/d for women and 1778 mg/d for men (Paturi *et al.* 2008); i.e. the average intake exceeds the dietary reference intake over 2-fold in women and 3-fold in men. These P intakes are in accord with other Western countries, as the average diet in European countries provides 1000-1500 mg P daily (EFSA 2005). Similar dietary P intakes are also common in other countries (e.g. Calvo 1993, Takeda *et al.* 2002, Gronowska-Senger and Kotanska 2004). In addition, some individuals have an excessively high P intake, being several grams per day. In Finland, differences exist in P intakes between regions and age groups, P intake being highest in the Oulu region and in the age group of 55-64 years in both sexes (Paturi *et al*. 2008). Although P is widely available virtually in all foods, P intake from dairy, grain and meat products covers 75% of the total average daily intake of P in Finnish diets (Paturi *et al.* 2008) (Table 5).

Table 5. *Contribution (%) of food groups to average daily intake of phosphorus among 25- to 64-year-old Finnish women and men.*

Food group	Women, % (mg/d)	Men, % (mg/d)
Dairy products	34 (459)	31 (558)
Grain products	28 (386)	30 (533)
Meat products	14 (196)	18 (317)
Fish	5 (64)	4 (76)
Vegetables	5 (70)	3 (52)
Others	13 (189)	13 (243)

Source: National FINDIET 2007 Survey (Paturi *et al.* 2008)

As the nutrition composition tables usually do not include P from phosphate additives, the intake of total P is underestimated (Oenning *et al.* 1988). In USA, the average dietary P intake from phosphate additives was estimated to be 470 mg/day per individual in 1990 (Calvo 1993). An earlier estimation suggested that phosphate additives contribute 20-30% of the adult total P intake (~320 mg/d) (Bell *et al.* 1977, Greger and Krystofiak 1982). However, already in the 1970s, Bell *et al.* (1977) calculated that if an individual continuously chooses foods with high P content, P intake could increase to 1 g/day.

2.1.2.2 Calcium

In Nordic countries, dietary Ca intake for adults is recommended to be 800 mg/d (Nordic Council of Ministers 2004). Finnish Nutrition Recommendations (National Nutrition Council 2005) are based on the Nordic Nutrition Recommendations. In the United States, adequate intake (AI) for Ca is 1000 mg/d for adults aged 19-50 years and 1200 mg/d for younger adults and the elderly (Food and Nutrition Board 1997). No global consensus of adequate daily Ca intake exists, and the dietary guidelines for Ca vary between countries. In Europe, the recommended Ca intake for adults varies from 700 mg/d to 1000 mg/d (see review by Bonjour *et al.* 2009b). Studies imply that daily Ca intake below 400 mg is insufficient. Evidence suggests that Ca supplementation offers the most important benefit for those individuals whose diet contain little Ca (Bonjour *et al.* 1997). The safe upper intake level (UL) for Ca is 2500 mg/d (Food and Nutrition Board 1997, National Nutrition Council 2005), and in the USA the lowest observed adverse effect level (LOAEL) has been set at 5000 mg/d (Food and Nutrition Board 1997).

In Finland, the main food sources of Ca are dairy products, which account for 71-75% of the total Ca intake from foods (Paturi *et al.* 2008) (Table 6). As the consumption of dairy products is high in Finland, the intake of Ca meets nutritional recommendations (800 mg/d) at a population level; according to the FINDIET 2007 Survey, the mean intake of Ca was 1007 mg/d in women and 1202 mg/d in men aged 25-64 years (Paturi *et al.* 2008). Unlike in Scandinavian countries, Ca intake in many other countries remains below recommended levels (e.g. Hendrix *et al.* 1995, Bryant *et al.* 1999, Guèguen and Pointillart 2000, Lombardi-Boccia *et al.* 2003, Salamoun *et al.* 2005). However, also in Finland, certain groups (e.g. vegan, lactose-intolerant and milk-allergy) may fail to achieve recommended levels. Moreover, the FINDIET-2002 Survey revealed that Ca intake was below dietary guidelines for women who drank no milk or soured milk (Männistö *et al.* 2003).

Table 6. *Contribution (%) of food groups to average daily intake of calcium among 25- to 64- year-old Finnish women and men.*

Food group	Women, % (mg/d)	Men, % (mg/d)
Dairy products	71 (713)	75 (902)
Fruit and berries	6 (58)	4 (44)
Beverages	6 (59)	5 (58)
Vegetables	4 (38)	3 (30)
Others	13 (139)	13 (168)

Source: National FINDIET 2007 Survey (Paturi *et al.* 2008)

2.1.2.3 Calcium-to-phosphorus ratio of diets

In 1975, the Life Science Research Office (LSRO) in USA concluded in their report: "Although there is a difference of scientific opinion, it is the opinion of the Selected Committee that the Ca:P ratio of the diet is important, especially if it varies substantially from 1 owing to the relatively high intake of phosphorus" (LSRO 1975). Since then, speculation has arisen whether the dietary Ca:P ratio is clinically significant in human adults (Food and Nutrition Board 1997, Sax 2001). However, in infants and children, a dietary Ca:P weight ratio of 1.5 is considered ideal for optimal growth, as this is the Ca:P ratio of human milk (EFSA 2005). This ratio also corresponds to the Ca:P ratio in the human bone mineral hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$).

In many Western countries, P intake is 2- to 3-fold above the dietary guidelines (Gregory *et al.* 1990, Takeda *et al.* 2002, Gronowska-Senger and Kotanska 2004, Paturi *et al.* 2008), whereas Ca intake remains below the recommendations (e.g. Henrix *et al.* 1995, Bryant *et al.* 1999, Guéguen and Pointillart 2000, Lombardi-Boccia *et al.* 2003, Salamoun *et al.* 2005) despite increased food fortification with Ca (Whiting and Wood 1997, Heaney *et al.* 2005) and Ca supplementation (Kim *et al.* 2003, Radimer *et al.* 2004). The overall trend in food consumption in Europe (Urho and Hasunen 1999, Comite´ de Nutrición de la Asociación Española de Pediatría 2003) as well as in the USA (Calvo and Park 1996, Harnack *et al.* 1999, Nielsen and Popkin 2004) is to drink less milk and more phosphoric acid-containing soft drinks, which in turn results in a lower dietary Ca:P ratio. If a habitual diet lacks dairy products, the dietary Ca:P ratio will easily drop below the optimal level. Moreover, the increased consumption of processed foods containing phosphate additives or the increased use of P-containing supplements will lead to a low dietary Ca:P ratio. Based on the latest dietary Ca and P intakes, the average Ca:P weight ratio (mg:mg) in the habitual diets of Finnish women aged 25-64 years is 0.74 and in men 0.68 (Paturi *et al.* 2008). These ratios are below the suggested weight Ca:P ratio of 1.3. In the age group of 65-74 years, the dietary Ca:P ratio is even lower, being 0.69 among women and 0.64 among men.

2.1.3 Bioavailability

2.1.3.1 Phosphorus

P is readily available in a wide range of foodstuffs and bioavailable from several foods. With dietary intakes of 775–1860 mg/d, 60-80% of P will be absorbed in the gut (Favus *et al.* 2006). The absorption rate is greatest in the jejunum, although absorption occurs throughout the small intestine. Most P absorption occurs by passive diffusion along an electrochemical gradient, but some also by saturable active transport across the cells by the luminal Na-phosphate-cotransporter type 2b (NPT2b) (Berner *et al*. 1976). NPT2b is stimulated by the active form of vitamin D ($1,25(OH)_2D$)(Chen *et al.* 1974, Katai *et al.*

1999). The absorption efficiency of P does not vary with dietary intake; thus, absorption is efficient with all P intakes. In addition, vitamin D is not an essential determinant for P absorption (Wilz *et al.* 1979, Williams and DeLuca 2007), although it increases P absorption to a certain extent. Absorption by the active mechanism is used only when P intake is low or the requirement for P is highly increased (Peterlik and Wasserman 1978). The absorbed amount of P is determined by the P content of the diet, bioavailability of P from foodstuffs and presence of natural P binders in foods. Pharmacological P binders are commonly used in kidney patients (Barton *et al*. 2009). High intake of dietary Ca (Spencer *et al.* 1984) can form insoluble salts with P, thus reducing P absorption.

Evidence has emerged that some forms of dietary P are less bioavailable. Although total P per g of protein is similar in animal products and plants (~20 mg/g protein) (Massey 2003), in plants most of the P (~75%) is in the form of phytate, which is poorly digested (for review, see Uribarri and Calvo 2003). Therefore, less P is absorbed from foods unless the food is processed with the enzyme phytase, e.g. leavening bread with yeast-producing phytase. The calculated total P content of grain products may be high, but the bioavailable P (soluble P) content is considerably lower. In fact, this was noted in recent food analyses measuring soluble P content of foodstuffs (Itkonen *et al.* 2009, Karp *et al.* 2009a). In these studies, P analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS) using an *in vitro* method (Ekholm *et al.* 2003). In foods, such as meat, poultry and fish, P is found mostly as intracellular organic compounds (amino acids, phospholipids, nucleotides), from which it is released during digestion. In milk, P is in different fractions and has different bioavailability from each of them, e.g. casein contains phosphopeptide, which is resistant to enzymatic hydrolyses (Uribarri and Calvo 2003). The absorbability of P in Ca-containing dairy products is not well known, although in the management of renal disease Ca compounds are used to bind dietary P (Nolan and Qunibi 2003).

While enormous variation exists in the bioavailability of P from natural P sources, P as a form of phosphate additives has been suggested to be almost 100% absorbed (Uribarri and Calvo 2003). It is noteworthy, that the bioavailability of dietary P sources has not been investigated in humans; all data concerning the bioavailability of P are based on extrapolation from animal studies, as summarized by Uribarri (2007). The only study examining bioavailability of P in humans showed that P originating from phosphate additives and P from meat increased S-Pi concentration and urinary phosphate (U-Pi) excretion more than P from whole-grain products in an acute controlled situation (Karp *et al.* 2007), indicating higher bioavailability of P from such food sources. Recent food analyses support this finding, as soluble P in phosphoric acid-containing soft drinks, which include phosphate additives, were revealed to be around 100% of total P content (Karp *et al.* 2009a). The form of phosphate additives might have different bioavailability, as absorption of P was observed to be more efficient from ortophosphates than from polyphosphates in males, suggesting that polyphosphates are not immediately hydrolysed and absorbed (Zemel and Linkswiler 1981).

2.1.3.2 Calcium

In humans, around 20-45% of dietary Ca is absorbed, mostly in the upper part of ileum, by passive diffusion or active energy-, and vitamin D-requiring processes. Unlike absorption of P, Ca absorption is dependent on vitamin D supply (Wilz *et al.* 1979). $1,25(OH)_2D$ increases the number of calcium binding proteins (CaBP) in mucosal cells. With adequate vitamin D status, the number of CaBP will increase and active Ca absorption will be optimal (DeLuca 1979). With poor vitamin D status, Ca absorption occurs only by passive diffusion, which cannot ensure adequate Ca status in the human body. Contrary to P, the ingested amount of Ca affects the absorbability rate; a higher Ca intake decreases and a lower Ca intake increases Ca absorption. Heaney *et al.* (1990) reported that with a 15-mg Ca load fractional absorption was 64% and with a 500-mg load 29%. Therefore, ingesting Ca over several meals throughout the day is more advisable than one large dose consumed in a single meal (Kärkkäinen *et al.* 2001). With increasing age, Ca absorption decreases (Heaney *et al*. 1989, Weaver *et al.* 1995), while during pregnancy (Heaney *et al.* 1989), puberty and infancy absorption increases. When necessary, a human body can adapt to lower Ca intakes, as has been demonstrated in people living in developing countries (e.g. Prentice 2007).

In the gastrointestinal tract, Ca must be released from food components into its free form to become soluble. The bioavailability of Ca from different foods varies. Ca bioavailability from dairy products (milk and cheese) is better than from spinach or sesame seeds (Kärkkäinen *et al.* 1997). In fact, Ca bioavailability from spinach and sesame seeds seems to be quite poor (Heaney *et al.* 1988, Kärkkäinen *et al.* 1997). The absorbability of Ca from kale has been found to be even higher than from milk (Heaney and Weaver 1990). Oxalate (found in spinach and beans) and phytate (found in unleavened bread, raw beans, seeds, nuts and grains) decrease Ca absorption (Weaver *et al.* 1987, Heaney *et al.* 1988, Heaney and Weaver 1989, Heaney *et al.* 1991, Charoenkiatkul *et al.* 2008), while no satisfactory evidence exists as to whether lactose affects Ca absorption (Schuette *et al.* 1991, Zitterman *et al.* 2000). In Ca supplements, Ca is mostly in form of Ca carbonate ($CaCO_3$) (DiSilvestro 2005), although absorbability of Ca from $CaCO_3$, as compared with other supplements (e.g. Ca citrate or Ca citrate malate), is not the highest (Nigar and Pak 1985, Miller *et al.* 1988).

2.1.4 Metabolism of phosphorus

2.1.4.1 Phosphorus in the human body

After oxygen, hydrogen, carbon, nitrogen and Ca, P is the 6th most abundant element in the human body. A 70-kg man has approximately 700 g of P in his body. Around 80-85% of the P is located in the skeleton as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The remaining P is located in extracellular fluids and soft tissues, mainly as a component of proteins,

phospholipids, nucleotides and nucleic acids (Fig. 1). Besides being an essential nutrient in bone mineralization, P has many other vital functions in the human body; it is involved in energy metabolism, cellular signalling through phosphorylation and is a structural part of phospholipids, nucleotides and nucleic acids (for review, see Berner and Shike 1988). Intracellular phosphate is present in a variety of phosphorylated compounds, such as adenosine triphosphate (ATP) and guanosine triphosphate (GTP), which are fundamental in energy metabolism and enzyme activation. Phosphorus also serves as an extra- and intracellular buffer through the interconversion of HPO_4^{2-} and $H_2PO_4^-$, thus helping to maintain normal pH.

In living tissues, P exists in the form of phosphate (PO_4^{3-}). Most of the P in whole blood is in the phospholipids of red blood cells and plasma lipoprotein, and only ~1 mmol/l is found as inorganic Pi, which can be in different forms, the most common being HPO_4^{2-} (Fig. 1). Inorganic Pi is measurable by laboratory measurements from plasma or serum samples. This fraction is an exchange pool between organs containing P (intestine, bone, kidneys and cells), regulating P homeostasis.

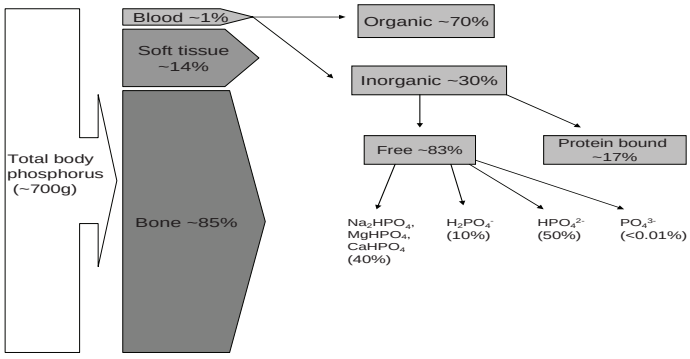


Figure 1. *Distribution of phosphorus in the human body (modified from Berner and Shike 1988).*

2.1.4.2 Phosphorus homeostasis and status

Homeostasis

The main P homeostasis regulation sites are the gastrointestinal tract (absorption organ), kidneys (excretion organ) and bone (storage organ). The most important regulation occurs in the kidneys, and homeostasis is achieved by excreting P in urine. In healthy humans with normal dietary P intake, around 6-7 g of P is filtered daily by the kidneys. More than 80% of P is reabsorbed in the proximal tubule and ~10% in the distal tubule and ~10% is excreted in urine. The predominant regulators of renal tubular Pi reabsorption are dietary P intake and S-PTH concentration (for review, see Murer *et al.* 1999, Berndt *et al.* 2007). Phosphorus in the human body is in balance when the output (loss of P in urine, faeces and sweat) is equal to the absorbed amount of P (net intestinal absorption) (Fig. 2).

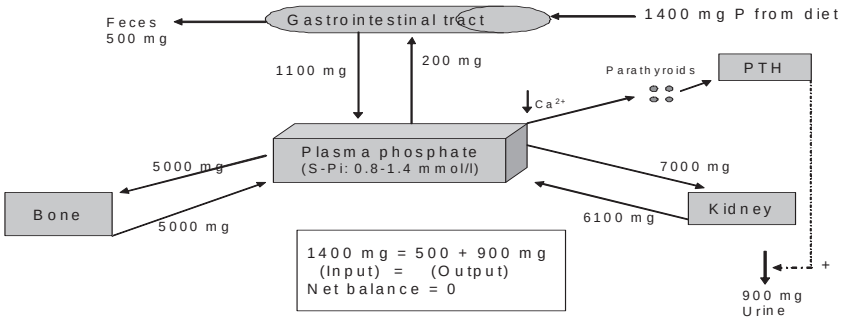


Figure 2. *Phosphorus homeostasis and balance in a normal adult (modified from Strain and Cashman 2005).*

S-Pi concentration is kept within the normal range mainly by parathyroid hormone (PTH) and 1,25(OH)$_2$D (Berndt and Kumar 2008). When S-Pi decreases, serum 1,25(OH)$_2$D (S-1,25(OH)$_2$D) increases, which elevates P absorption in the gut and release of P from bone. The elevated serum PTH (S-PTH) concentration increases U-Pi excretion and release of P from bone (Fig. 3). The effect of 1,25(OH)$_2$D on U-Pi excretion is indirect; an increase in serum calcium (S-Ca) concentration, which is mediated by 1,25(OH)$_2$D, suppresses PTH secretion, which, in turn, enhances Pi reabsorption (Berner and Shike 1988, Shimada *et al.* 2004a). As discussed in the reviews of Berndt and Kumar (2008) and Quarles (2008), newly discovering signalling pathways involving P regulation have been presented; a key role in P metabolism has been suggested for Na-phosphate-cotransporters (NPTs), which are found in different tissues (for review see Biber *et al.* 2009). Type 1 and 2 NPT proteins have been observed in the kidneys. A high-P diet decreases while a low-P diet increases the number of NPT2a in the proximal tubule, the former increasing U-Pi excretion (Keusch *et al.* 1998).

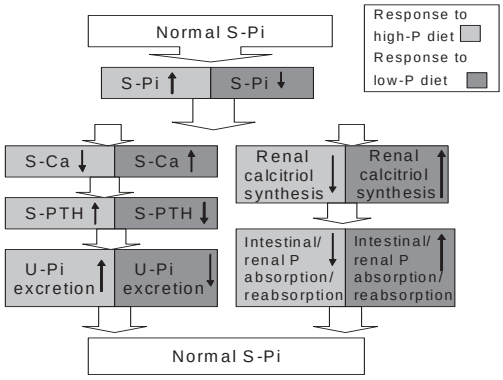


Figure 3. *Regulation of phosphorus (P) metabolism in situations with high and low dietary P intakes in healthy humans (modified from Berndt and Kumar 2008).*

Nutritional phosphorus status

No specific marker that reflects whole-body P status has been identified. As the major determinants of S-Pi concentration are dietary P intake, intestinal absorption of P, U-Pi excretion and shift of P into cells, by measuring S-Pi concentration and U-Pi excretion,

information on whole-body P homeostasis is gained. Normal U-Pi excretion ranges from 20 to 50 mmol/24 h, while normal S-Pi of fasting serum samples ranges from 0.85 to 1.65 mmol/l (Yhtyneet Medix Laboratoriot 2009). U-Pi excretion reflects dietary P intake (Fig. 3), and S-PTH concentration, which is the main regulator of P metabolism, also gives information about P homeostasis. However, as dietary P intake directly affects S-Pi concentration and hypo- and hyperphosphataemia result in dysfunction or disease, the American Food and Nutrition Board (1997) considered S-Pi the best indicator of nutritional adequacy of dietary P in adults. Dietary P intake has been observed to influence S-Pi concentration when followed over 24 h (Portale *et al.* 1989, Calvo *et al.* 1991, Kärkkäinen and Lamberg-Allardt 1996). Diurnal variation exists in S-Pi concentrations, S-Pi being the lowest in the morning (8:00-11:00) and the highest during the night (Portale *et al.* 1987, Touitou *et al.* 1989). A low S-Pi concentration leads to rickets in children and osteomalacia in adults. By contrast, a high S-Pi concentration results in hyperphosphataemia, which is a severe condition in renal disease patients.

2.1.4.3 Disturbances in phosphorus metabolism

Abnormalities in any major determinants of S-Pi can result in hypo- or hyperphosphataemia. A dietary-induced P deficiency is very unlikely, as P is widely available in various foods. Instead, hypophosphataemia arises from poorly managed parenteral nutrition, diabetic ketoacidosis or other severe diseases (Lotz *et al.* 1968, Berner and Shike 1988, Crook 2009). When S-Pi concentration is <0.32 mmol/l, symptoms of hypophosphataemia (e.g. muscle dysfunction and weakness, disorders of the central nervous system) can be observed (Weisinger and Bellorin-Font 1998).

In clinical hyperphosphataemia, renal failure is usually the most common cause. When kidney function is reduced, U-Pi excretion decreases and more P remains inside the body, leading to hyperphosphataemia (Bushinsky 2001, Uribarri 2007, Sullivan *et al.* 2009). When a patient suffers from only mild or moderate renal failure, an increase in S-PTH concentration will compensate P retention, as PTH is the principal regulator correcting hyperphosphataemia. However, when renal failure is severe, S-Pi typically rises to levels well above the normal reference range (S-Pi >1.50 mmol/l) (Välimäki 2000, Yhtyneet Medix Laboratoriot 2009). An acute rise in S-Pi leads to a direct increase in PTH secretion and a decrease in the renal hydroxylation of calcidiol (25(OH)D) to 1,25$(OH)_2$D in the kidney (Fig. 3). A decreasing S-1,25$(OH)_2$D reduces Ca absorption and results in hypocalcaemia and elevated PTH secretion. Elevated S-PTH concentration is typically found in patients with end-stage renal disease (Goodman 2001). It is noteworthy that a decrease in S-1,25$(OH)_2$D results in additional PTH secretion at any level of S-iCa (Bushinsky 2001). In early renal failure, decreased S-1,25$(OH)_2$D and S-iCa contribute to increased secretion of PTH. However, when renal disease progresses, impaired 1,25$(OH)_2$D production and low S-iCa levels resulting from impaired Ca absorption stimulate PTH secretion and promote parathyroid gland hyperplasia (overgrowth of the parathyroid gland due to an increased number of cells) (Komaba *et al.* 2008). Secondary

hyperparathyroidism (accelerated function of the parathyroid gland) is a common finding in these circumstances (Martin *et al.* 2005). In chronic renal failure, alterations in Ca and P homeostasis may lead to a bone disease previously known renal osteodystrophy but recently renamed chronic kidney disease mineral and bone disorder (CKD-MBD) (Komaba *et al.* 2008, Bover *et al.* 2009) (Fig. 4). Treatment of secondary hyperparathyroidism is essential in the management of CKD-MBD. To prevent hyperphosphataemia, patients with renal disease use P binders to reduce dietary P absorption (Nolan and Qunibi 2003) and limit their consumption of foods with high P content (Sullivan *et al.* 2009).

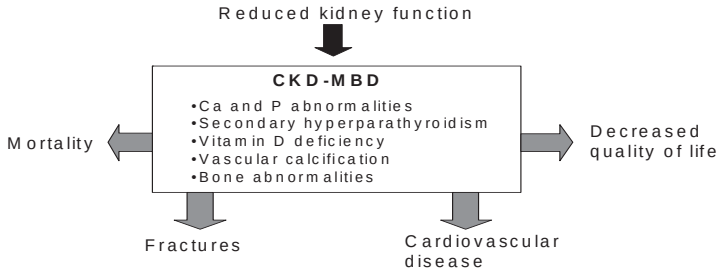


Figure 4. *Consequences of chronic kidney disease mineral and bone disorder (CKD-MBD) in patients with chronic kidney disease (adapted from Komaba et al. 2008).*

2.1.4.4 Effects of dietary phosphorus on phosphorus metabolism

When S-Pi is followed over a 24-h period in healthy humans, it closely reflects dietary P intake (Portale *et al.* 1987, Portale *et al.* 1989, Calvo *et al.* 1991), but in some studies, when only the fasting S-Pi has been measured, no clear effects of the ingested P on S-Pi have been observed (Food and Nutrition Board 1997). Dietary P restriction, in turn, decreased S-Pi concentration in healthy humans, leading to increased $1,25(OH)_2D$ production in kidneys (Maierhofer *et al.* 1984, Portale *et al.* 1989). Different foods might also vary in their effects on S-Pi due to differences in the bioavailability of P. Karp *et al.* (2007) found that cheese, which contains more Ca than most other foods studied (phosphate salts, meat and whole grain products), increased S-Pi markedly. The authors presented several possible mechanisms behind this increase, but they could not conclusively explain this finding. While foods might vary in their effects on S-Pi, also the effects of natural P and P from phosphate additives on S-Pi might differ. In studies containing small numbers of participants, foods with P additives increased S-Pi (Bell *et al.* 1977) more than foods containing natural P (Karp *et al.* 2009b, unpublished data).

Animal studies have widely demonstrated that both U-Pi and faecal P excretion increase with high P intake (e.g. Shah and Meranger 1970, Katsumata *et al.* 2004, Tani *et al.* 2007). Increased S-Pi concentration has independently been shown to elevate PTH secretion *in vitro* (Slatopolsky *et al.* 1996, Naveh-Many *et al.* 1999) and decrease $1,25(OH)_2D$ production in animals (Zhang *et al.* 2002) and humans (Portale *et al.* 1986, Portale *et al.*

1987). Elevated S-PTH concentration is known to increase U-Pi excretion; therefore, a high dietary P intake has unsurprisingly increased U-Pi excretion in healthy women and men in intervention studies (Bell *et al.* 1977, Calvo *et al.* 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996, Whybro *et al.* 1998). However, in kidney patients U-Pi excretion was decreased due to impaired renal function even after a high dietary P intake (Bushinsky 2001, Slatopolsky *et al.* 2001). In addition, foods with a high P content are usually high in protein. Protein intake has been found to be associated with increased U-Pi excretion, although results have differed according to the age group in humans (Lakshmanan *et al.* 1984). Oestrogen may also affect U-Pi excretion by suppressing sodium-dependent P reabsorption in the kidneys (Uemura *et al.* 2000). A recent study suggests that ethnic differences may exist in U-Pi excretion in response to oral P administration (Yan *et al.* 2009). After P loading in healthy 60- to 75-year-old females and males, the Chinese had more rapid renal clearance of P than Gambian or British subjects.

2.1.4.5 Effects of dietary calcium on phosphorus metabolism

Most of the ingested Ca remains in the gut lumen, where it can bind other nutrients such as P. Heaney and Nordin (2002) presented that each 500 mg of ingested Ca binds 166 mg of dietary P. Ca capacity to bind P in the gastrointestinal tract is exploited when treated kidney patients, as Ca supplements are widely used as P binders (for review, see Nolan and Qunibi 2003). In intervention studies where Ca intake has been adequate (1000 mg) or high (1995 mg), high P intake has not increased S-Pi concentration significantly (Whybro *et al.* 1998, Grimm *et al*. 2001). Ca infusion (Howard *et al.* 1953, Nordin and Fraser 1954) as well as oral Ca intake in the form of $CaCO_3$ (Yang *et al.* 1994, Mortensen and Charles 1996) or Ca gluconate (Spencer *et al.* 1984) diminished U-Pi excretion in both healthy females and males, but not in hypoparathyroid patients (Howard *et al.* 1953, Nordin and Fraser 1954). In healthy humans, this is due to increased S-iCa concentration, which in turns decreases S-PTH concentration and leads to lower U-Pi excretion, even without any alterations in P intake. In addition, high Ca intake increases the formation of Ca-Pi complex in the intestine (Mortensen and Charles 1996). In the same study, the consumption of milk increased U-Pi excretion in 28- to 59-year-old women, although milk has a high Ca content. This was explained to be due to the high-P content of milk. This finding is in accord with a recently conducted 24-h controlled study, in which milk increased U-Pi excretion more than a Ca supplement ($CaCO_3$) or Ca-enriched mineral water in 14 healthy young females (Kemi *et al.* 2009a).

2.1.4.6 Effects of dietary calcium-to-phosphorus ratio on phosphorus metabolism

No studies have specifically investigated the effects of dietary Ca:P ratios on P metabolism, but in some intervention studies with healthy humans, low Ca:P ratios, i.e. diets with high P and low Ca, affected P metabolism by increasing S-Pi concentration and

PTH secretion. These in turn, elevated U-Pi excretion in acute (Kärkkäinen and Lamberg-Allardt 1996), 8-day (Calvo *et al.* 1988) and 4-week situations (Calvo *et al.* 1990).

2.1.5 Metabolism of calcium

2.1.5.1 Calcium in the human body

In the adult human body, Ca content comprises approximately 1000 g in women and 1200 g in men. Most of it (>99%) is located in the skeleton and the teeth as hydroxyapatite. The remaining Ca is found in blood, extracellular fluid, muscle and other tissues and cells. Besides being an elemental part of hydroxyapatite in bones, Ca is an important regulator of several body functions: intracellular signalling, muscle contraction, functioning of the nervous system, hormone and enzyme secretion and blood clotting. Therefore, the concentration of both intra- and extracellular Ca is tightly regulated. In serum, around 50% of Ca is in ionised form (S-iCa), and the other 50% is bound to serum protein, mainly in albumin and globulines (Favus and Goltzman 2008). This bound form of Ca is not biologically active, unlike the ionised form. The amount of Ca bound to proteins increases with increasing serum albumin and alkaline pH. As a result of normal daily bone turnover, around 500 mg of Ca is released from bone and and the same amount is accreted (Fig. 5). Excess absorbed Ca that cannot be stored in bone is excreted in urine, faeces and sweat, while non-absorbed calcium is excreted in faeces (Charles *et al.* 1991). The relation between dietary Ca and Ca loss depends on intestinal Ca absorption efficiency, skeletal turnover and balance and U-Ca excretion in the kidneys, as endogenous and dermal Ca loss remain low. In bone, 99.5% of Ca is in the form of insoluble hydroxyapatite, and only 0.5% is released by resorption or deposited during bone formation.

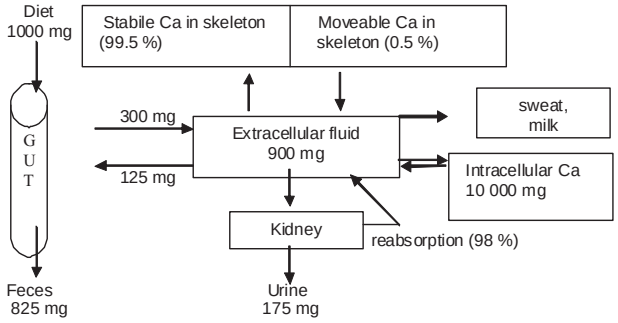


Figure 5. *Distribution of calcium in the human body with a diet containing 1000 mg of Ca (modified from Välimäki 2000).*

2.1.5.2 Calcium homeostasis and status

Homeostasis

Ca homeostasis is tightly regulated by PTH, $1,25(OH)_2D$ and calcitonin in the intestine, bone and kidney (Fig. 6). PTH and $1,25(OH)_2D$ are secreted when S-iCa is low, and calcitonin, when S-iCa is high. In humans, the most important regulators are PTH and $1,25(OH)_2D$. Ca-sensing receptors exist in parathyroid and kidney cells (Brown *et al.* 1993, Brown and Lian 2008). These receptors sense very small reductions in S-iCa concentrations, which, in turn, cause an increase in PTH secretion (Brown and Hebert 1997). In response to low S-iCa concentration, PTH secretion increases rapidly (Schmitt *et al.* 1996), and $1,25(OH)_2D$ is produced more in the kidneys. These actions lead to increased Ca absorption and decreased U-Ca excretion, the end result of which is a rise in S-iCa concentration to normal levels (Fig. 6). In addition, PTH and $1,25(OH)_2D$ act together to mobilize Ca^{2+} from bone to serum (for review, see Holick 1996). PTH secretion decreases due to a feedback mechanism induced by increased $1,25(OH)_2D$ and S-iCa. Recent findings in mice suggest that Na-phosphate-cotransporter (NPT) type 2c may also maintain normal Ca metabolism, probably by modulating the vitaminD/FGF-23 axis (Segawa *et al.* 2009).

Adequate vitamin D status in the human body is vital for Ca homeostasis, as $1,25(OH)_2D$ plays an essential role in Ca metabolism by increasing Ca absorption in the gut and by increasing bone resorption, leading to increased S-iCa concentration. The S-$1,25(OH)_2D$ concentration rises in response to decreased Ca intake (Dawson-Hughes *et al.* 1993), decreased S-Ca concentration and increased S-PTH concentration (Boden and Kaplan 1990). In the kidneys, PTH enhances 1-α-hydroxylaze activity, thus inducing conversion of 25(OH)D to the $1,25(OH)_2D$ (Garabedian *et al.* 1972) (Fig. 6).

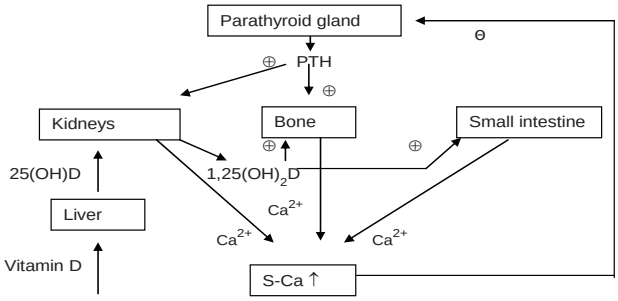


Figure 6. *Effects of parathyroid hormone and calcitriol on calcium metabolism. + represents stimulative effects and – preventive effects (modified from Välimäki 2000).*

With normal dietary Ca intakes (~1000 mg/d), around 10 g of Ca is filtered daily through the kidneys and more than 98% of this is reabsorbed (Favus and Goltzman 2008). PTH is the most important regulator of U-Ca excretion, and an increase in S-PTH concentration decreases Ca excretion into urine by increasing tubular reabsorption of Ca (Lajeunesse *et*

al. 1994). Nonetheless, some dietary components such as protein (Hegsted *et al.* 1981, Lakshmanan *et al.* 1984, Kerstetter *et al.* 2003), sodium (Nordin *et al.* 1993, Evans *et al.* 1997) and caffeine (Harris and Dawson-Hughes 1994) increase U-Ca excretion. However, dietary protein has recently been shown to enhance Ca absorption, thus offsetting U-Ca loss (Kerstetter *et al.* 2005). Vitamin D supplementation also increases U-Ca excretion, presumably by increasing Ca absorption (Mortensen and Charles 1996). Understandably, high dietary Ca intake increases U-Ca excretion (e.g. Matkovic *et al.* 1995, Hill *et al.* 2008), whereas high dietary P (e.g. Hegsted *et al.* 1981, Kärkkäinen and Lamberg-Allardt 1996) and potassium (Lemann *et al*. 1993, Rafferty *et al.* 2005) intakes decrease absorption.

Nutritional calcium status

As the concentrations of intra- and extracellular Ca are tightly regulated, and Ca, when needed, is available from the skeleton, assessment of Ca status is complicated. No specific marker exists for assessing Ca status of individuals or populations (Weaver 1990). In healthy individuals, S-Ca is rarely ever low due to Ca deficiency. As S-Ca is tightly controlled and kept within a narrow range, S-Ca poorly reflects total body Ca. S-Ca concentration is normally between 2.15 and 2.51 mmol/l, while serum ionized Ca (S-iCa) concentration is maintained at 1.18-1.30 mmol/l at a pH of 7.33-7.43 (Yhtyneet Medix Laboratoriot 2009). S-iCa, the physiologically active Ca in serum, functions as an intracellular Ca regulator, and S-iCa concentration is strictly regulated and follows a circadian rhythm (Markowitz *et al.* 1988, Calvo *et al.* 1991). S-Ca concentration has a similar circadian rhythm to S-iCa (Markowitz *et al.* 1988). Minisola *et al.* (1993) found that S-iCa concentration decreases with age in men, but not in women. In addition, Calvo *et al.* (1991) reported sex differences in the nocturnal adaptation to fasting, as women had lower S-iCa concentrations and higher U-Ca excretions after at 05:00. In follow-ups of 24 h or less, oral Ca intake (dose 500–1500 mg) increased S-iCa and decreased S-PTH concentrations as well as increased U-Ca excretion in both men and women (Herfarth *et al.* 1992a, Horowitz *et al.* 1994, Kärkkäinen *et al.* 2001).

As S-iCa concentration is maintained at normal levels, by inducing increases in PTH secretion, S-PTH concentration gives useful information about Ca homeostasis when measured together with S-iCa, S-Ca and U-Ca. In fact, PTH response to an oral Ca load has been used as an indicator of Ca bioavailability from Ca supplements (Gonnelli *et al.* 1995) and foods (Kärkkäinen *et al.* 1997). In a review discussing how to assess Ca status, the author proposes that urinary Ca/creatinine (Cr) ratios might be a useful tool for assessment of Ca status, even from 2-h fasting urine samples (Weaver 1990), as U-Ca is significantly, albeit with a low correlation, related to Ca intake. In research, U-Ca excretion has been used as a marker of Ca absorption, although U-Ca excretion does not equal the amount of absorbed Ca (Mortensen and Charles 1996). Normal 24-h U-Ca excretion is 1.25-5.50 mmol (Yhtyneet Medix Laboratoriot 2009). Calvo *et al.* (1991) demonstrated diurnal variation in U-Ca excretion, with a decrease at nighttime.

2.1.5.3 Effects of dietary calcium on calcium metabolism

Although Ca absorption rate decreases with increasing Ca intake (Heaney *et al.* 1989), oral Ca intake has in several studies been found to increase S-iCa concentration in healthy men and women of varied ages (e.g. Herfarth *et al.* 1992b, Kärkkäinen *et al*. 2001, Sadideen and Swaminathan 2004). Oral Ca intake (dose ≥172 mg) has been demonstrated to acutely (within hours) suppress PTH secretion (e.g. Herfarth *et al.* 1992a, Kärkkäinen *et al.* 1997, Guillemant *et al*. 1994, Guillemant *et al.* 2000, Kärkkäinen *et al.* 2001). In fact, acute dose-dependent effects on S-iCa and S-PTH concentrations after 250- and 1000-mg Ca doses (Kärkkäinen *et al.* 2001) as well as after 500- and 1500-mg Ca doses (Guillemant and Guillemant 1993) have been reported. However, with administration of a single oral 1000-mg and 2000-mg Ca dose, S-iCa increased in a similar manner, with the maximal increase occurring after 2 h of Ca administration, indicating saturation of the active Ca absorption mechanism (Herfarth *et al.* 1992a). When Ca intake was diminished from 900 to 170 mg/d for four days, S-PTH increased from 24 to 41 ng/l in premenopausal women (Prince *et al.* 1990). With age, the increasing effects of Ca load on S-iCa and the decreasing effects on S-PTH have been observed to diminish (Guillemant *et al.* 1994). Understandably, as in normal physiological conditions, an increase in S-PTH concentration results in lower U-Ca excretion, an increase in U-Ca excretion is found in response to higher dietary Ca intake (Harvey *et al.* 1988, Guillemant and Guillemant 1993, Matkovic *et al.* 1995, Kärkkäinen *et al.* 2001). In a controlled situation, U-Ca excretion strongly correlates with acute Ca intake (for review, see Charles 1992). With ageing, U-Ca excretion decreases (Davis *et al.* 1970), which might be due to an age-related decrease in Ca absorption, a reduction in the filtered Ca amount or a poor vitamin D status. In addition, Ca intake has indirect effects on $1,25(OH)_2D$, as PTH, the central regulator of Ca metabolism, mediates the impact of Ca intake on $1,25(OH)_2D$; elevated S-PTH increases the production of $1,25(OH)_2D$ in the kidneys. S-PTH and S-25(OH)D concentrations correlate negatively (e.g. Lamberg-Allardt *et al.* 2001), but S-$1,25(OH)_2D$ does not correlate with S-PTH, although S-$1,25(OH)_2D$ is an important down-regulator of PTH synthesis.

2.1.5.4 Effects of dietary phosphorus on calcium metabolism

Earlier studies imply that dietary P might interfere with Ca metabolism in several ways: by directly affecting S-iCa concentration (Herfarth *et al.* 1992b) and U-Ca excretion (Lau *et al.* 1982) or through PTH secretion (Kilav *et al.* 1995) and $1,25(OH)_2D$ production (Yoshida *et al.* 2001). Conflicting results exist concerning the effects of P on Ca absorption (Spencer *et al.* 1978, Zemel and Linkswiler 1981, Heaney and Recker 1982, Heaney 2000), and only a few studies have been conducted on this topic, usually with a small number of subjects. An increase in dietary P intake (2000 mg/d) increased faecal Ca excretion in some but not all study subjects, when daily Ca intake was ≥2000 mg, but not when Ca intake was < 1500 mg (Spencer *et al.* 1978). No association was present between Ca absorption efficiency and P intake in women of different age groups (Heaney and

Recker 1982, Heaney 2000). Different phosphate additives may vary in their effects on Ca absorption, as polyphosphates have decreased Ca absorption compared with ortophosphates (Zemel and Linkswiler 1981). While it is uncertain whether P directly affects Ca absorption, P might influence absorption through $1,25(OH)_2D$ synthesis, as P directly and independently determines the $1,25(OH)_2D$ production rate by affecting the function of 1-α-hydroxylase *in vivo* (Yoshida *et al.* 2001). These effects have been demonstrated also in healthy humans; P supplementation (3000 mg/d) for 10 days decreased S-$1,25(OH)_2D$ concentration, whereas P restriction (500 mg/d) increased the concentration (Portale *et al.* 1986). Based on the findings of Portale and co-workers (1984, 1986, 1987, 1989), P regulates the production rate of $1,25(OH)_2D$, thus affecting the S-$1,25(OH)_2D$ concentration. In postmenopausal women, the association between S-PTH and S-$1,25(OH)_2D$ was significant only with a moderate dietary P intake, but the association diminished with high or low P intakes (Dawson-Hughes *et al.* 1991). However, in the same age group of healthy women, S-$1,25(OH)_2D$ was no affected by high P intake despite elevated S-PTH concentration (Silverberg *et al.* 1989). In osteoporotic women, acute P intake decreased S-$1,25(OH)_2D$ levels (Silverberg *et al.* 1989).

In an intervention study, dietary P supplementation (3000 mg/d) for 10 days decreased S-iCa concentration in healthy males (Portale *et al.* 1987). In some studies, a high P intake was reported to even decrease S-Ca concentration in both sexes (Reiss *et al.* 1970, Bell *et al.* 1977, Silverberg *et al.* 1986). The mechanism underlying the impact of high P intake on S-iCa remains unclear, but S-iCa may decrease due to diminished Ca absorption as a result of formation of the Ca-Pi complex in the gut. While S-iCa and S-Ca decreased after P loading in humans, it has also been demonstrated that P *per se* increases PTH secretion *in vitro* (Slatopolsky *et al.* 1996) and *in vivo* in rats (Kilav *et al.* 1995), probably through the NTPs in parathyroid glands (Tatsumi *et al.* 1998, Miyamoto *et al.* 1999). Strong evidence has emerged in animals that high-P diets increase PTH secretion (for review, see Calvo and Park 1996). Masuyama *et al.* (2000) found in rats that a high-P diet reduces PTH action in the kidneys, despite the increased S-PTH concentration, by decreasing the number of PTH receptors. In some studies with humans, high dietary P intake increased S-PTH concentration in longer term situations (e.g. Portale *et al.* 1986, Silverberg *et al.* 1986), but no studies have properly investigated the dose-response effects of dietary P intakes. In addition, P sources may differ in their effects on S-PTH; acutely, P originating from phosphate additives alone increased S-PTH concentration more than P from cheese, meat and whole-grain products (Karp *et al.* 2007).

In normal physiological conditions, elevated S-PTH decreases U-Ca excretion. Dietary P might also directly affect Ca reabsorption in the kidneys by enhancing reabsorption independently of PTH, S-Ca and renal Na handling (Lau *et al.* 1982). Although a high-P diet decreases U-Ca excretion (Hegsted, *et al.* 1981), there is no evidence that P significantly affects faecal Ca excretion. When dietary P intake was increased from 800 to 2000 mg/d at varied dietary Ca intake levels (200, 800 and 2000 mg/d), Spencer *et al.* (1984) noted only a slight increase in the faecal Ca excretion rate, while U-Ca excretion

decreased significantly. The different forms of phosphate salts may also vary in their responses to Ca metabolism. In rats fed a high-P diet, the development of nephrocalcinosis and diminished kidney function was more severe with P ingested in the form of polyphosphates than in the form of ortophosphates (Matsuzaki *et al.* 1999).

2.1.5.5 Effects of dietary calcium-to-phosphorus ratio on calcium metabolism

In studies with mice, rats and dogs, a low dietary Ca:P ratio increased PTH secretion in a chronic manner (Shah *et al.* 1967, Clark 1969, Krook *et al.* 1971, Koshihara *et al.* 2005a, Huttunen *et al.* 2007). Moreover, S-Ca level in rats seemed to be more dependent on dietary Ca:P ratio than on the absolute dietary Ca intake (Clark 1969). In humans, only two studies conducted over 50 years ago with a low number of subjects specifically investigated the effects of dietary Ca:P ratios on Ca metabolism (Leichsenring *et al.* 1951, Patton *et al.* 1953) (Table 7). More recent intervention studies have evaluated only high-P, low-Ca diets (Calvo *et al.* 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996) or high-P, adequate-Ca/high-Ca diets (Whybro *et al.* 1998, Grimm *et al.* 2001) (Table 7). Patton *et al.* (1953) described U-Ca excretion to increase with an increasing Ca:P ratio and a constant Ca intake. At varying levels of Ca intake, when P intake was increased, no significant effect on Ca retention was observed. However, when P intake was kept constant, an increase in Ca intake resulted in an increase in Ca balance. In intervention studies, low-Ca, high-P diets decreased S-iCa and increased S-PTH concentration in healthy young men and women (Calvo *et al.* 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996). In these studies, hormonal changes (increased S-PTH) similar to those observed in animal studies (for review, see Calvo and Park 1996) were demonstrated, suggesting the adverse effects of low dietary Ca:P ratios on Ca metabolism. Although dietary Ca intake was adequate (800 mg), by increasing the daily P intake from 800 to 1800 mg S-PTH increased (Whybro *et al.* 1998). However, when Ca intake was high (1995 mg/d), high P (~3000 mg/d) intake had no effect on S-PTH (Grimm *et al.* 2001). A diet with high P and low Ca may cause alterations also in other Ca-regulating hormones, as Calvo and co-workers (1990) found that after a 4-week low-Ca, high-P diet S-PTH levels increased, but no changes occurred in S-1,25$(OH)_2$D concentration, which usually increases in response to low Ca intake.

Table 7. *Studies investigating the effects of dietary Ca:P ratios or high-P diets.*

Study	Ca intake (mg/d)	Total P intake: diet + dose (P dose) (mg/d)	Weight Ca:P ratio (molar ratio)	Duration of diet	*Number of subjects (sex)**
Leichsenring *et al.* 1951	300 1500 1500	800 800 1400 (600)	0.38 (0.28) 1.87 (1.40) 1.06 (0.80)	4 weeks	*17 (F)*
Patton *et al.* 1953	344 944 1544	766, 1066 (300), 1366 (600) 766, 1066 (300), 1366 (600) 766, 1066 (300), 1366 (600)	0.45(0.34),0.32 (0.24),0.25(0.19) 1.23(0.93),0.89 (0.67),0.69(0.52) 2.01(1.52),1.45 (1.09),1.13(0.85)	2 weeks	*18 (F)*
Calvo *et al.* 1988	420	1660	0.25(0.19)	8 days	*8 (F) +8 (M)*
Calvo *et al.* 1990	400	1700	0.24 (0.18)	4 weeks	*15 (F)*
Kärkkäinen and Lamberg-Allardt 1996	375	2378 (1500)	0.16 (0.12)	24 hours	*10 (F)*
Whybro *et al.* 1998	1000	1000 (0) 2000 (1000), 2500 (1500), 3000 (2000)	1.0 (0.75) 0.50 (0.38) 0.40 (0.30) 0.33 (0.25)	1 week	*11 (M)*
Whybro *et al.* 1998	800	1800 (1000)	0.44 (0.33)	1 week	*9 (M)*
Grimm *et al.* 2001	1995	3008 (1595)	0.66 (0.50)	6 weeks	*10 (F)*

* F=female, M=male

2.2 Bone

Two types of bone tissue exist: cortical bone, which is the main form of bone tissue, and trabecular bone. Trabecular bone is metabolically more active. It has a shorter remodelling cycle (3 months), while in cortical bone it lasts 4 months (Dempster 1995). Bone tissue components include organic matrix, cells and minerals, which are mostly in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Bone has three types of cells: bone-forming cells (osteoblasts), bone-resorbing cells (osteoclasts) and osteocytes (for review, see Raisz 2005). In the adult skeleton, 90-95% of bone cells are osteocytes, 4-6% osteoblasts and 1-2% osteoclasts (see Bonewald 2008). Bone organic matrix contains approximately 90% type I collagen and 10% different non-collagenous proteins (Robey and Boskey 2008).

2.2.1 Osteoporosis

Osteoporosis and osteoporotic fractures are considered major public health problems in developed countries and are costly worldwide (Kannus *et al.* 1999, International Osteoporosis Foundation 2004). The definition of osteoporosis is "a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk" (Anon 1993). The risk of fracture can be predicted by measuring bone mineral density (BMD) (Marshall *et al.* 1996), and a BMD value of -2.5 standard deviations (SD) or lower in relative to young adults is defined as osteoporosis (WHO 1994). The primary diagnostic technique for measuring BMD is dual-energy x-ray absorptiometry (DXA), but other techniques are also available, as summarized by Bonjour *et al.* (2009a).

Osteoporosis is becoming an increasingly severe disease in the ageing societies of Western countries. Osteoporosis affects an estimated 75 million people in Europe, USA and Japan (EFFO and NOF 1997). In Europe, the number of osteoporotic fractures in 2000 was estimated to be 3.79 million, 0.89 million of which were hip fractures (Kanis and Johnell 2005). This estimation suggests that in a population aged over 50 years, one in three women and one in five men will suffer osteoporotic fractures (Melton *et al.* 1992, Melton *et al.* 1998, Kanis *et al.* 2000). The most severe osteoporotic fracture is a hip fracture, which usually occurs in an elderly person after a fall. In both women and men, ageing and low BMD are the major risk factors for osteoporotic fractures. Due to lower peak bone mass, earlier and greater bone loss and longer life span, osteoporosis and osteoporotic fractures are more common among women than men. In Finland, at the end of the 20th century, Kannus *et al.* (1999) predicted that the incidence of osteoporotic fractures would increase 3-fold by 2030, based on the incidence of hip fractures from 1960 to 1994. However, more recent calculations suggest that these estimations may be too high (Kannus *et al.* 2006), as the incidence of fractures followed between 1997 and 2004 declined by 17% in women and 6% in men. The authors speculated that this might be due to a healthier ageing population, an increase in body weight and improved functional ability in the elderly. It might also be due to better screening and treatment for osteoporosis and fractures.

2.2.1.1 Effects of lifestyle factors on risk of osteoporosis

Peak bone mass (PBM) is an essential determinant in the risk of osteoporotic fractures in later life. Genes determine 60-80% of bone mass (Nguyen *et al.* 1998, Hunter 2005). Lifestyle factors, such as physical activity (Welten *et al.* 1994), nutrition (see reviews by Robins and New 1997, Bonjour *et al.* 2009b), smoking (Law and Hackshaw 1997, Nevitt *et al.* 2005,), alcohol consumption (Hernandez-Avila *et al.* 1991, García-Sanches *et al.* 1995), diseases (e.g. malabsorption, anorexia, hypogonadism) and the use of certain medicines, also affect bone mass. Moreover, nutrients and genes interact with each other, as reviewed by Gillies (2003). Several nutrients affect bone health at different stages of

life. These nutrients also play a potential role in osteoporosis prevention (for review, see Bonjour *et al.* 2009b). The most investigated nutrient is Ca, and the importance of both adequate vitamin D status and Ca intake in bone health are well established (Welten *et al.* 1995, Bischoff-Ferrari *et al*. 2005, Tang *et al*. 2007). As presented in a review by Bonjour *et al.* (2009b), other nutrients, e.g. P, magnesium (Mg), vitamin K and strontium, as well as protein (for review, see Ginty 2003) also influence bone health.

Childhood and adolescence are vitally important periods; bone mass accumulates until the age of 20 (Theintz *et al*. 1992, Kröger *et al.* 1993) or even until the age of 30 (Recker *et al.* 1992), when PBM is gained. During growth, heredity, specific nutrient intakes (vitamin D, Ca, P, protein), endocrine factors (sex steroids, IGF-I, $1,25(OH)_2D$), physical activity and body weight influence bone mass accumulation (see review by Bonjour *et al.* 2009a). Adequate Ca intake has been recognized to be an important determinant of PBM (for review, see Flynn 2003). Ca is considered the limiting factor for bone mineral accrual, and Ca intake of 1300 mg/d is proposed to fulfil the retention rates in puberty (Bailey *et al.* 2000). As summarized by Heaney (2009) and Bonjour *et al.* (2009a), some but not all studies conducted during childhood and adolescence have noted a positive correlation between dietary Ca intake, mainly derived from dairy products, and bone mineral mass. Vitamin D is required for normal skeletal growth, and deficiency results in rickets in children. The impact of vitamin D status or supplementation on bone measures in children has been limited despite suppressive effects on S-PTH (Bonjour *et al.* 2009a). Instead, physical activity during growth enhances bone accrual (Välimäki *et al*. 1994, Uusi-Rasi *et al.* 1997, Heinonen *et al.* 2000, MacKelvie *et al.* 2004). Activity in childhood is associated with adult BMD (Pesonen *et al.* 2005).

While bone mass is gained in childhood, in adulthood bone mass is maintained and its loss should be prevented. Bone loss is a normal physiological process, occuring in all humans in response to hormonal changes and decreased physical activity levels and muscle mass. However, as presented in a review by Bonjour *et al.* (2009a), if an individual gains high PBM in childhood, it will decrease the risk of osteoporotic fractures in later life; an increase in PBM by one SD will reduce the fracture risk by 50%. In postmenopausal women, bone loss is due to the anabolic effect of oestrogen depletion. By the age of 80 years, women are estimated to lose 30-50% and men 25-30% of bone mass (Väänänen 1996). In epidemiological and intervention studies, Ca intake and BMD in women correlated positively before (Welten *et al.* 1995) and after menopause (Reid *et al.* 1995, Shea *et al.* 2004). In adults, strong evidence links insufficient vitamin D status, as determined by low S-25OHD concentrations, to the development of osteoporosis (Zitterman 2003).

2.2.2 Bone metabolism

As a living tissue, bone renews itself continuously. Briefly, in this remodeling process, osteoclasts remove old bone and osteoblasts form new bone (for review, see Raisz 2005).

Several nutrients are important; Ca, P and Mg are needed for bone matrix formation, while sufficient vitamin D status ensures active Ca absorption in the gut. Protein and some minerals are also needed for collagen synthesis (for review, see Bonjour *et al.* 2009b). Bone remodelling is regulated by hormones and local factors (Table 8). The most important hormones regulating remodelling are PTH and $1,25(OH)_2D$. In healthy adults, a balance exists between the functions of osteoclasts and osteoblasts in ensuring skeletal maintenance and integrity. When bone turnover increases in women due to oestrogen withdrawal in menopause, bone loss accelerates due to increased bone remodelling; thus more bone is resorbed than replaced (for review, see Seeman 2002). Secondary hyperparathyroidism might further increase remodelling in both elderly men and women, as reduced Ca absorption decreases S-Ca concentration, which in turn increases PTH secretion to ensure the maintenance of S-Ca. This is done by increased cortical bone remodelling (Seeman 2002), thus resulting in Ca and P release from bone.

Table 8. *Regulation of bone remodelling (adapted from Raisz 1999).*

Factor	Bone resorption	Bone formation
PTH[1]	↑	↑ (↓)*
$1,25(OH)_2D$[1]	↑	↑ (↓)*
Calcitonin	↓	?
Oestrogen	↓	(↓)**
Growth hormone/IGF[1]	↑	↑
Thyroid hormone	↑	↑
Glucocorticoids	↑***	↓

[1] PTH, parathyroid hormone; $1,25(OH)_2D$, calcitriol; IGF, insulin like growth factor
↑ = increase, ↓ = decrease, ?= not known
* PTH and vitamin D decrease collagen synthesis in high doses
** Decreases bone formation by decreasing remodelling, but formation is decreased less than resorption and bone mass increases
*** May increase resorption indirectly by inhibiting Ca absorption and sex hormone production

2.2.2.1 Regulators of bone metabolism

Parathyroid hormone

The parathyroid gland synthesizes PTH. The biologically active PTH form (intact PTH) is a polypeptide containing 84 amino acids. PTH is secreted in response to relatively small changes in S-iCa concentrations. The effects of S-iCa concentrations on the parathyroid gland are mediated by extracellular Ca-sensing receptors (Brown *et al.* 1993). Intact PTH is degraded rapidly in the liver (70%) and kidneys (20%), as the half-life of intact PTH is only ca. 2 min (Schmitt *et al.* 1996), while the half-lives of inactive PTH forms are ca. 45 min (Herfarth *et al.* 1992b). Ca supplementation has been found to decrease PTH secretion more in younger (20-40 years) than older (60-88 years) individuals (Guillemant *et al.* 1994). As discussed earlier, high S-iCa and S-$1,25(OH)_2D$ concentrations produce feedback inhibition for PTH secretion, while high S-Pi increases PTH secretion. The latest results from an animal study suggest that FGF-23 directly inhibits PTH secretion (Ben-

Dov *et al.* 2007). In addition, transmembrane protein α-klotho, which FGF23 needs in the kidney to inhibit U-Pi reabsorption, is also found in parathyroid cells. α-klotho may mediate the effects of S-Pi on PTH secretion (Brownstein *et al.* 2008).

PTH is a major regulator of bone metabolism, but it has dual effects on bone; intermittent administration of PTH stimulates bone formation (Liu and Kalu 1990) and increases trabecular bone mass (Hodsman *et al.* 1991), while continuous excessive PTH secretion, which is common especially in hyperparathyroidism, increases bone turnover (Tam *et al.* 1982, Schiller *et al.* 1999) and releases Ca and P from bone. Thus, continuously high S-PTH leads to decreased bone mineral mass. Since intermittent administration of PTH has been found to be favourable for bone, it is used in combination with oestrogen for osteoporosis treatment in women after menopause (Lindsay *et al.* 1997). PTH has direct effects on bone through PTH receptors in osteoblasts (Talmage *et al.* 1976), and with high continuous PTH concentrations there is an acute inhibition of collagen synthesis (Dietrich *et al.* 1976). Ca released from bone has been hypothesized to also directly regulate osteoblasts and osteoclasts, as some evidence has emerged that osteoblasts and osteoclasts may sense extracellular Ca concentrations (Quarles 1997).

PTH concentration increases with age in both females and males (Endres *et al.* 1987, Chan *et al.* 1992, Minisola *et al.* 1993, Khaw *et al.* 1994), which may produce an increase in bone turnover and a loss of bone mass, particularly in cortical bone (for review, see Raisz 1999). There is also a diurnal variation in PTH secretion; S-PTH concentration is the highest at 18:00 and at 02:00 (Calvo et al. 1991) and apparently lower in the mornings at 09:30-10:00 (Calvo *et al.* 1991, Herfart *et al.* 1992b). The difference between peak and nadir is around 30% (Logue *et al.* 1990). S-PTH concentration follows changes in S-iCa (Markowitz *et al.* 1988, Calvo *et al.* 1991, Schmitt *et al.* 1996) as well as S-Pi concentrations (Markowitz *et al.* 1981*,* Portale *et al.* 1984, Herfarth *et al.* 1992b).

Calcitriol

The main effect of vitamin D on bone is mediated through Ca balance, as the $1,25(OH)_2D$ increases Ca absorption in the gut and in cooperation with PTH increases the release of Ca and P from bone. $1,25(OH)_2D$ independently interacts with the vitamin D receptor in the parathyroid glands, resulting in an inhibition of PTH gene transcription (for review, see Holick 1996). VDRs have been found in more than 30 different tissues, e.g. bone, gut and parathyroid glands (for review, see Zitterman 2003). Based on the present knowledge, the main function of vitamin D on bone is to maintain a healthy mineralized skeleton by ensuring sufficient blood and extracellular Ca and Pi concentrations. Changes in vitamin D status (S-25OHD) are mediated through PTH.

FGF-23

FGF-23, a 32-kDa protein, has a specific role in P and bone metabolism. The kidney is a principal target organ for FGF-23. Osteoblasts and osteocytes produce FGF-23 in response to the increased S-Pi and $1,25(OH)_2D$ concentrations (Kolek *et al.* 2005, Saito *et al.* 2005). Serum FGF-23 concentration (S-FGF23) increases when S-Pi increases (Yu and White

2005). In the kidneys, FGF-23 inhibits Pi reabsorption, thus promoting U-Pi excretion, and also inhibits $1,25(OH)_2D$ production (Shimada *et al.* 2001, Shimada *et al.* 2004a). It has been reported that an increase in S-FGF-23 predicts an increase in S-PTH (Kazama *et al.* 2005). In healthy humans, none or only modest alterations occur in S-FGF-23 concentrations in response to dietary P intakes, while in animals, a low-P diet decreases and a high-P diet increases S-FGF-23 concentrations as summarized by Shaikh (2008). FGF-23-null mice have decreased BMD, elevated S-Pi and $1,25(OH)_2D$ concentrations and low S-PTH concentrations (Shimada *et al.* 2004b). However, it is unknown whether the decreased bone mineralization is a direct effect of the decreased FGF-23 or due to the elevated S-Pi and $1,25(OH)_2D$ concentrations. Many unsolved questions remain, e.g. how and where S-Pi changes are sensed in the human body and how these changes lead to different S-FGF-23 levels. It is also unclear how the production of FGF-23 is controlled (for reviews, see Fukumoto 2008, Bergwitz and Jüppner 2010).

Oestrogen

Oestrogen is essential for normal epiphyseal maturation and skeletal mineralization in puberty in girls and boys. Oestrogen also regulates bone turnover throughout life in both sexes (see review by Raisz 2005). Oestrogen deficiency leads to increased bone remodelling, seen as a more efficient resorption than formation rate and decreased bone mass. This most commonly occurs in postmenopausal women. In menopause, oestrogen treatment decreases bone turnover by acting directly on bone cells through their specific oestrogen receptors. The results of an *in vitro* study suggest that oestrogen may regulate PTH indirectly, possibly via FGF-23 (Carrillo-López *et al*. 2009). Oestrogen replacement therapy decreased S-PTH concentration in postmenopausal women (Khosla *et al.* 1997).

Some evidence suggests that the use of combined hormonal contraceptives preserves bone mass in perimenopausal women (Martins *et al.* 2006). However, the use of contraceptives during adolescence has been associated with lower BMD (Martins *et al.* 2006). Recently, the use of oral hormonal contraceptives and the duration of use were found to be associated with lower BMD in 19- to 30-year-old women (Scholes *et al.* 2010). Hormonal contraceptives suppress ovarian oestrogen production, keeping the circulating oestrogen concentration low. This might be the mechanism causing deficits in bone mass in adolescence. This is especially seen with the use of injectable contraceptives (depot medroxy-progesterone acetate, DMPA), which is rarely used among women in Finland (Backman *et al.* 2008). DMPA has been found to be associated with lower BMD and increased bone resorption (Ott *et al.* 2001, Scholes *et al.* 2005), as the use of DMPA produces a hypo-oestrogenic state in women. However, women who discontinued DMPA use gained BMD (Curtis and Martins 2006).

2.2.2.2 Markers of bone metabolism

Bone remodelling, also known as bone turnover, is an essential part of bone health. A typical remodelling cycle includes 7-10 days of resorption and 2-3 months of formation.

In remodelling, around 10% of bone is replaced every year (for review, see Watts 1999). Biomarkers of bone remodelling are classified into markers of bone resorption (Table 9) and formation (Table 10). Today, numerous novel and specific bone markers are available, as presented in the review by Seibel (2002). Thus, the short-term effect of nutrients and other lifestyle factors on bone metabolism *in vivo* can be monitored, by measuring markers from serum and urine samples. Some markers specifically measure certain phases of the remodelling cycle and others reflect general turnover rate. As most marker components are also present in tissues other than bone, non-skeletal processes may have an influence on them. One such marker is serum total alkaline phosphatase (ALP), which is a widely used bone remodelling marker. Unlike its isoenzyme bone alkaline phosphatase (BALP), ALP is not bone-specific, as it originates also from the liver, intestine, kidney and placenta. When measuring bone markers, the large intra- and interindividual variability in the concentrations of bone markers should be taken into account.

Table 9. *Biochemical markers of bone resorption (modified from Seibel 2002).*

Marker	Process	Origin	Specimen
CTx*	Bone resorption	All tissues containing type I collagen	Serum, urine
DPD*	Bone resorption	Bone, dentin	Serum, urine
Hypro*	Bone resorption	Bone, cartilage, soft tissue, skin	Urine
NTx*	Bone resorption	All tissues containing type I collagen	Serum, urine
PYD*	Bone resorption	Bone, cartilage, tendon, blood vessels	Serum, urine
TRACP 5b*	Osteoclast number	Osteoclasts, bone, blood	Plasma, serum

* CTX, carboxyl-terminal telopeptide of collagen type I; DPD, deoxypyridinoline; Hypro, hydroxyproline; NTx, aminoterminal telopeptide of collagen type I; PYD, pyridinoline; TRACP 5b, tartrate-resistant acid phosphatase 5b.

Table 10. *Biochemical markers of bone formation (modified from Seibel 2002).*

Marker	Process	Origin	Specimen
BALP*	Osteoblast differentation	Bone	Serum
OC*	Bone formation	Bone, platelets	Serum
PICP*	Bone formation	Bone, soft tissue, skin	Serum
PINP*	Bone formation	Bone, soft tissue, skin	Serum

* BALP, bone-specific alkaline phosphatase; OC, osteocalcin; PICP, C-terminal propeptide of type I collagen; PINP, N-terminal propeptide of type I collagen.

2.2.3 Effects of dietary phosphorus on bone

According to several animal and limited human intervention studies, a high P intake affects bone metabolism through alterations in Ca, PTH and $1,25(OH)_2D$ metabolism (for review see Calvo and Park 1996). Mainly, the effects of P on bone metabolism are

mediated through the increased PTH secretion. In healthy humans, a continuously high S-PTH results in higher bone resorption and release of Ca and P from bone, while the intermittent administration of PTH has the opposite effects. In fact, teriparatide, the N-terminal (1-34) fragment of recombinant human PTH is used to treat osteoporosis (for review, see Hodsman *et al.* 2005). 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. However, when renal function is impaired, excess P intake has very damaging effects on bone, as chronic kidney disease might lead to a multifactorial bone disorder, CKD-MBD (for review, see Leonard 2009). Previously, Lundquist *et al.* (2007) reported that *in vitro* bone mineralization by P is dependent on osteoblast NPT2 transporters, as osteoblasts expressed both NPT2a and NPT2b, which are responsible for the majority of osteoblast P uptake, in addition to NPT3 type. Unlike results from *in vivo* studies, high doses of P *in vitro* have prevented bone resorption by reducing the formation of osteoclasts and the activity of mature osteoclasts (Yates *et al.* 1991).

Secondary hyperparathyroidism is known to negatively affect bone health by increasing bone remodelling. In experimental rats, Katsumata *et al.* (2005) and Huttunen *et al.* (2006, 2007) reported that diets high in P resulted in secondary hyperparathyroidism and bone loss. In healthy humans, no controlled or follow-up studies exist on the effects of different P doses on bone mass, structure or geometry. However, evidence from an epidemiological cross-sectional study suggests that greater than recommended P intakes are negatively and independently associated with lower amounts of bone mass in young women (Metz *et al.* 1993). P sources might also vary in their effects on bone, as some earlier epidemiological studies have revealed unfavourable associations of phosphoric acid-containing soft drinks with bone (Fernando *et al.* 1999, Wyshak 2000, Tucker *et al.* 2006). 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. sodium polyphosphates, are used (Suurseppä *et al.* 2001). Although P content of cola beverages is low and does not contribute a large P propotion of the total P intakes in normal diets, when cola beverages are consumed in high quantities, e.g. over 1.5 l/d, such an amount may contribute notably to the total P intake. It is unclear whether it is phosphoric acid or some other component in phosphoric acid-containing beverages that negatively affects bone (Tucker *et al.* 2006). The designs of some of these studies have been criticized (Anderson 2001). In addition, cola beverages contain phosphate additives alone, unlike other foods, which usually contain only natural P or both. Two earlier intervention studies indicated that P from phosphate additives alone (Karp *et al.* 2007) or phosphate additives in foods (Bell *et al.* 1977) might have more negative effects on bone than natural P in foods.

2.2.4 Effects of dietary calcium on bone

The importance of adequate Ca intake for BMD in children and adults has been widely evaluated and established (see e.g. reviews by Heaney 2009, Bonjour *et al.* 2009a). Studies in children and adolescents indicate that those who receive Ca supplementation

(Bonjour *et al.* 2009a) or have higher dietary Ca intake (Wosje and Specker 2000) gain greater BMD. During the growth period adequate Ca intake can maximize the positive effects of physical activity on bone health (Specker and Binkley 2003). Nieves *et al.* (1995) predicted that by increasing Ca intake from 800 to 1200 mg/d during teenage years, hip BMD would increase by 6%. However, the skeleton might be more responsive to Ca supplementation before the beginning of pubertal maturation than during the peripubertal period (Wosje and Specker 2000). Epidemiological studies indicate that high Ca intake through the lifetime could decrease fracture risk even by 60% (Heaney 1992). Meta-analyses concluded that in the postmenopausal period Ca supplementation has positive effects on BMD by maintaining bone mass (Welten *et al.* 1995) and attenuating bone loss (Shea *et al.* 2002). Beneficial effects of Ca supplementation on BMD might also be possible later in life, as summarized by Flynn (2003).

The associations between the consumption of dairy products and bone health have been widely investigated (for review, see Guéguen and Pointillart 2000, Heaney 2009). Dairy product consumption has been positively associated with BMC and BMD in several randomized controlled and observational studies during varying periods of life (Bonjour *et al.* 2009a, Heaney 2009). In addition, a recent meta-analysis in children concluded that the BMC of the total body and lumbar spine were increased with higher Ca intake and dairy product consumption (Huncharek *et al.* 2008). A retrospective study in women aged 20-49 years suggested that milk consumption in childhood and adolescence might be positively related to bone mineral mass and inversely to the risk of fractures (Kalkwarf *et al.* 2003). However, contradictory results of the association between dairy products consumption and fracture risk also exist; for example, a meta-analysis of nearly 40 000 subjects indicated that low milk consumption was not associated with any marked increase in fracture risk (Kanis *et al.* 2005).

The short-term effects of Ca administration (Ca supplement or Ca-enriched mineral water) on the markers of bone resorption and formation have been investigated in healthy adults. Ca administration decreased the concentration of several bone resorption markers (U-PYD, U-DPD, U-CTx, U-NTx, S-NTx, S-CTx, S-ICTP) (Horowitz *et al.* 1994, Guillemant *et al.* 2000, Villa *et al.* 2000, Guillemant *et al.* 2003, Guillemant *et al*. 2004, Sadideen and Swaminathan 2004) or had no effects (S-ICTP, U-DPD) (Kärkkäinen *et al.* 2001). Conflicting results of the effects of Ca intake on bone formation markers have reported in some studies, although relatively few studies exist in this field. In young women, Ca restriction (<250 mg/d) increased S-OC concentration (Kusuhara *et al.* 1991), while Ca supplementation acutely (Kärkkäinen *et al.* 2001) or for two weeks (Ginty *et al.* 1998) did not affect S-OC, S-BALP or S-PICP concentrations. However, S-PICP concentration increased after a 7-day Ca intake (800 mg/d), which was followed by a 22-day low Ca intake (<300 mg/d) period (Åkesson *et al.* 1998).

2.2.5 Effects of dietary calcium-to-phosphorus ratio on bone

The adverse effects of low Ca:P ratios in animal diets are quite convincing, as animals fed with high-P and low-Ca, i.e. low Ca:P ratio, diets manifested secondary hyperparathyroidism (seen as e.g. increased S-PTH and decreased S-Ca concentrations), loss of bone and osteopenia (for review, see Calvo and Park 1996). Bell and co-workers (1980) found that by increasing Ca intake of mature mice the adverse effects of high P intake on bone could be partially diminished. Mice receiving a low-P diet with varying Ca intakes had higher bone weight and mineral content than mice with a high-P diet with varying Ca intakes. A few years ago, Koshihara *et al.* (2005b) reported that a high Ca:P ratio due to low P intake was favourable for bone mineralization in adult rats since it increased Ca absorption. A reduction in dietary Ca:P ratio, in turn, decreased bone mass and strength in oestrogen-deficient rats (Koshihara *et al.* 2005a). Some cross-sectional studies in humans have also described an association between dietary Ca:P ratios and BMC or BMD. A positive correlation was noted between dietary Ca:P ratio and BMD in perimenopausal women (Lukert *et al.* 1987) and between dietary Ca:P ratio and BMC in older men, but not in older women (Yano *et al.* 1985). In a more recent cross-sectional study, Basabe *et al.* (2004) concluded that high Ca intake (1000 mg/d) and a Ca:P weight ratio exceeding 0.74 were associated with better BMD in young females. These findings are in accord with epidemiological studies in young females conducted in the 1990s by Metz *et al.* (1993) and Teegarden *et al.* (1998). Interestingly, the Ca:P ratio of a single foodstuff might affect bone metabolism, as the consumption of cheese, which has a high Ca:P ratio, decreased S-PTH and bone resorption (U-NTx), unlike the other P sources examined (phosphate salts, meat, whole-grain products) (Karp *et al.* 2007).

3 Aims of the study

The main objective of this thesis was to examine the effects of dietary phosphorus (P) on calcium (Ca) and bone metabolism in healthy Finnish women. Dietary P was evaluated since P intake is high in Finland, as in many other Western countries. In addition, the rising consumption of processed foods during the last decades has increased not only the intake of total dietary P but also P from phosphate-containing food additives. Moreover, some previous studies have suggested that excessive P intake could be deleterious to bone through increased parathyroid hormone secretion. Therefore, the specific aims here were to investigate P intakes commonly found in Western diets as well as to compare the effects of dietary P originating from natural P and from phosphate additives. As the metabolism of Ca and P is tightly bound together, not only the effects of dietary P *per se* but also the dual effects of Ca and P intakes on bone health were evaluated. Women served as study subjects since before menopause they are more vulnerable than men to developing osteoporosis due to a lower peak bone mass.

Specific research questions addressed were as follows:

Study I: Does dietary P affect Ca and bone metabolism in healthy females in a controlled short-term study? Are the effects of dietary P dose-dependent?

Study II: Do the effects of high P intake on Ca and bone metabolism diminish with increasing dietary Ca intake in healthy females in a controlled short-term situation? Are the effects of dietary Ca dose-dependent when dietary P intake is high?

Study III: Are associations of dietary P originating from natural P and P in phosphate additives with the central markers of Ca and bone metabolism different in healthy females in a cross-sectional study design? Are the associations between the central markers of Ca and bone metabolism and total high habitual dietary P intakes different from the low intakes?

Study IV: Are there relationships between dietary Ca:P ratios and serum parathyroid hormone concentration and Ca metabolism in the habitual diets of healthy females in a cross-sectional study? Is the optimal dietary Ca:P molar ratio of 1 achievable in habitual diets of healthy women whose dietary Ca intake is adequate?

4 Subjects and methods

4.1 Subjects

The participants were healthy 20- to 43-year-old Finnish females with no illnesses and using no medications known to affect calcium and bone metabolism, except hormonal contraceptives. In each study, women with irregular menstruation or no menstruation due to menopause were excluded. In addition, women with incomplete 4-day food records were excluded in Studies III and IV. Basic characteristics of the study subjects are presented in Table 11.

In Study I, 15 women aged 20-28 years were recruited from students of the University of Helsinki. In Study II, 15 women aged 20-40 years were recruited among both students and employees of the University of Helsinki. One woman in Study I discontinued the study because of severe headache, and three women in Study II for personal reasons.

Participants in Studies III and IV represent a randomly selected subgroup of 31- to 43-year-old Finnish women. Invited subjects had participated in the National FINRISK Study 1997 Survey, organized by the National Public Health Institute in the spring 1997 (FINDIET 1997 Study Group 1998). Subjects (aged 30-43 years) who had participated in the FINRISK Study were invited to this additional study in 1998. The final study group in Studies III and IV comprised 147 healthy premenopausal women.

Table 11. *Background characteristics and mean daily dietary energy and nutrient intakes (±SEM) of subjects in Studies I-IV.*

Variable	Study I (n=14)	Study II (n=12)	Studies III and IV (n=147)
Age (year, range)	20-28	20-40	31-43
Body mass index (kg/m^2)	20.9 (0.4)	22.2 (0.9)	23.3 (0.3)
Contraceptive users (%)	50.0	50.0	26.5
Energy intake (MJ/d)	7.9 (0.5)	7.7 (0.2)	7.9 (0.2)
Calcium intake (mg/d)	1134 (104)	883 (97)	1056 (34)
Phosphorus intake (mg/d)	1501 (73)	1247 (83)	1411 (33)
Protein intake (g/d)	67 (3)	64 (5)	73 (2)
Sodium intake (g/d)	3.1 (0.2)	2.8 (0.2)	1.9 (0.1)

4.2 Dietary data collection

In Studies I-IV, participants were given instructions both in writing and verbally to maintain their habitual food intakes during the period, in which a 4-day food record was kept, and to record all foods and beverages immediately after consumption. Three

weekdays and one weekend day were included in the 4-day food record. A researcher together with the participant checked the 4-day food record. In studies I and II, participants kept a 4-day food record before the first study session. Basic characteristics of typical dietary intakes of the participants in all studies are presented in Table 11.

The habitual dietary intake of the participants was calculated with computer-based programs, including Flamingo (version 0.5.6, Helsinki, 1999) in Study I, DIET 32 (version 1.22, Aivo Oy, Turku, Finland) in Study II and the Unilever Dietary Analysis Program (UNIDAP, Becel Palvelu Paasivaara Oy, Finland, 1989) in Studies III and IV. All of the programs are based on the food composition database (Fineli) of the Finnish National Institute for Health and Welfare (National Institute for Health and Welfare 2009).

4.3 Study designs and hypothesis

Studies I and II were controlled short-term (24-h) studies, and within these studies all study day meals were identical for each subject on each study day. No additional meals or snacks were allowed, but water was provided *ad libitum*. Study participants ate all meals, except supper, in the research unit. In both studies, meals were prepared from normal foods purchased from Finnish grocery stores and cooked and apportioned by the same person in the research unit on each study day. Controlled studies were performed with calcium (Ca) and phosphorus (P) doses, both normal and achievable, in Western diets. In addition, P and Ca were ingested throughout the day, simulating the situation in which food with high P or Ca content is ingested. Meals and supplements were always received after blood and urine sampling. The dietary contents of study day meals were calculated with the computer-based program Flamingo (version 0.5.6, Helsinki, 1999) in Study I and with DIET 32 (version 1.22, Aivo Oy, Turku, Finland) in Study II.

Studies III and IV were substudies of the same original cross-sectional study (Lamberg-Allardt *et al.* 2001). Data were collected during the visit and included fasting blood samples and the return of the 4-day food record and the self-reported questionnaire that was used to collect background information. Three separate 24-h urinary samples were collected during the study period from February to March 1998.

In each study, background information was collected with self-reported questionnaires. Both the weight and height of participants were self-reported in all studies.

4.3.1 Study I: High P intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females

In Study I, we hypothesized that higher P doses have more negative effects on Ca and bone metabolism than lower doses.

Fourteen young women attended and completed four 24-h sessions during a one-month period in April and May 2001. Each participant served as her own control. The participants were given, in randomized session order, 0 (placebo), 250, 750 or 1500 mg of P as a commonly used phosphate additive in the food industry (mixture of disodiumphosphate and trisodiumphosphate) (Six Oy, Helsinki, Finland) in 1000 ml of berry juice during the sessions. Juice with or without P was served in three equal-sized separate doses during the study meals. The serving times of meals and P doses are presented in Figure 7.

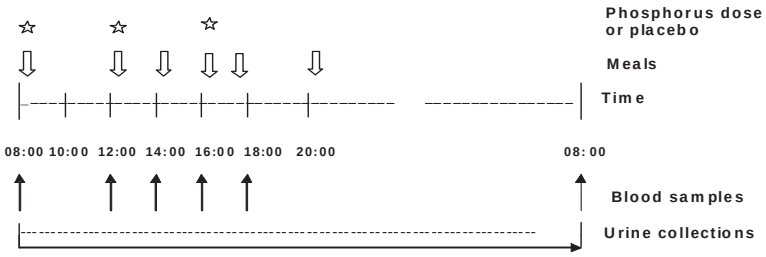


Figure 7. *Schedule of study days in Study I.*

The meals provided a total energy content of 8.4 MJ (2000 kcal), with a calculated Ca content of 250 mg and a P content of 495 mg. With the exception of P load, study days were otherwise identical. The P doses were chosen for the following reasons: P intakes of 495 mg (placebo) represented P intake below the recommended level (Food and Nutrition Board 1997), and 745 mg (250-mg P dose) represented P intake at the recommended level (Food and Nutrition Board 1997). The P intake of 1245 mg (750-mg P dose) corresponded to the mean P intake of Finnish females (Männistö *et al.* 2003), while the P intake of 1995 mg (1500-mg P dose) typifies the mean P intake of Finnish males (Männistö *et al.* 2003). The total P intakes and dietary calcium-to-phosphorus ratios (Ca:P ratios) of subjects throughout the study sessions are presented in Table 12.

Table 12. *Total phosphorus (P) intake of study subjects and dietary calcium-to-phosphorus ratios (Ca:P ratios) on study days in Study I.*

Variable	P dose 0 mg (placebo)	P dose 250 mg	P dose 750 mg	P dose 1500 mg
Total P intake (P dose + dietary P) (mg)	495*	745*	1245*	1995*
Ca:P weight ratio	0.51	0.34	0.20	0.13
Ca:P molar ratio	0.39	0.26	0.15	0.10

* Intakes of P and Ca from study day meals were 495 mg and 250 mg, respectively

4.3.2 Study II: Increased Ca intake does not completely counteract the effects of increased P intake on bone: an acute dose-response study in healthy females

Study II was a sequel to Study I. We hypothesized that by increasing Ca intake the effects of higher P intake on Ca and bone metabolism would be diminished.

Twelve women attended and completed three 24-h study sessions over a one-month period in March and April 2002. The participants were given 0 (placebo), 600 or 1200 mg of Ca as a Ca supplement (Ca carbonate) (Kalsium, Friggs, Oy Seege Ab, Helsinki, Finland) in 450 ml of diluted sugar-free lemon juice (Fun Light Lemon, Felix Abba Oy Ab, Turku, Finland) during each of the three sessions (Fig. 8). The subjects received the Ca supplement in three separate equally sized doses during the study day breakfast, lunch and dinner. The serving times of meals and Ca doses are presented in Figure 8. The order of the study sessions was randomized, and each subject served as her own control.

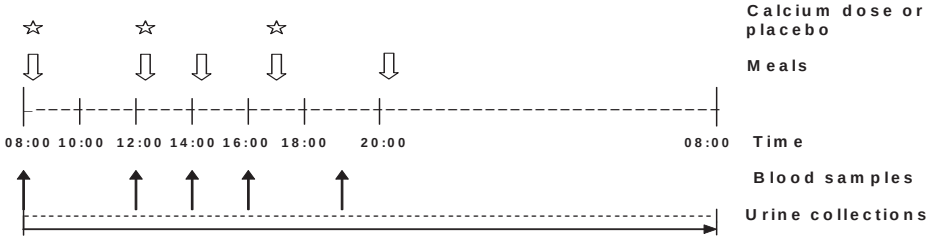


Figure 8 *Schedule of study days in Study II.*

The meals provided a total energy of 8.4 MJ (2000 kcal). With the exception of the Ca load, study days were otherwise identical. Study design included a control day, representing low Ca (480 mg/d) and a 2.5-fold higher P (1850 mg/d) intake than the current RDA (Food and Nutrition Board 1997), a 600-mg Ca dose day, representing adequate Ca (1080 mg/d) and a 2.5-fold higher P (1850 mg/d) intake than the current RDA (Food and Nutrition Board 1997) and a 1200-mg Ca dose day, representing high Ca (1680 mg/d) and P (1850 mg/d) intake 2.5-fold above the current RDA (Food and Nutrition Board 1997). In addition, the 600-mg dose session represents Ca intake corresponding to the average intake of Ca among Finnish females (Männistö *et al.* 2003). The total Ca intakes and the dietary Ca:P ratios of subjects throughout the three study days are presented in Table 13.

Table 13. *Total calcium (Ca) intake of study subjects and calcium-to-phosphorus-ratios (Ca:P ratio) on study days in Study II.*

Variable	Ca dose 0 mg (control)	Ca dose 600 mg	Ca dose 1200 mg
Total Ca intake (Ca dose + dietary Ca) (mg)	480*	1080*	1680*
Ca:P weight ratio	0.26	0.58	0.91
Ca:P molar ratio	0.20	0.45	0.70

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

4.3.3 Study III: Habitual high P intakes and foods with phosphate additives negatively affect serum PTH concentration: a cross-sectional study in healthy premenopausal women

In Study III, we hypothesized that high habitual dietary P intake would have more unfavourable associations than low intake with serum PTH concentration and Ca metabolism. In addition, we hypothesized that dietary P from phosphate additives is more negatively associated with S-PTH and Ca metabolism than natural P.

As dietary P can affect S-PTH directly (Slatopolsky *et al.* 1996) or through S-iCa (Herfarth *et al.* 1992b), the association of dietary P intakes and sources with both variables were investigated in this cross-sectional study. Participants were divided into quartiles based on their dietary P intakes. Only the extreme total P quartiles were evaluated; the quartiles situated between the extremes were ignored. Consequently, total P intakes could be assessed in a manner similar to a controlled study design (Study I). Quartiles sizes and P intake in quartiles are presented in Table 14.

In Finland, dairy products are the main sources of both dietary Ca and P. Therefore, to investigate the effects of natural P and phosphate-containing food additives, milk, cheese and processed cheese were chosen. Milk and cheese, excluding processed cheese, contain only natural P, and processed cheese contains both natural P and phosphate additives. To examine the relationship between natural P and phosphate additives and S-PTH and Ca metabolism, participants were divided into groups based on milk and cheese consumption and processed cheese consumption. 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 from milk and cheese. Because of the small number of consumers of processed cheese, participants were divided into two groups of unequal size (consumers and non-consumers). Group sizes and P intake from selected foods are presented in Table 14.

Table 14. *Quartiles and groups investigated in Study III.*

Quartiles and groups	Mean phosphorus intake (mg/d) (SEM)	Number of subjects
Total phosphorus 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 phosphorus from milk and cheese, excluding processed cheese
§= Phosphorus intake from milk and cheese, excluding processed cheese
¶= Phosphorus intake from processed cheese

4.3.4 Study IV: Low calcium:phosphorus ratio in habitual diets affects serum PTH concentration and Ca metabolism in healthy women with adequate Ca intake

In Study IV, we hypothesized that low habitual dietary Ca:P ratios have a more negative association than high ratios with serum PTH concentration and Ca metabolism.

In this cross-sectional study, participants were divided into quartiles based on their habitual dietary Ca:P ratio. Dietary Ca:P ratios were calculated based on the information of participants' dietary Ca (mg/d) and P (mg/d) intakes provided in the 4-day food record. Weight Ca:P ratios were converted into molar ratios by using molecular weight of Ca (40.08 g/mol) and P (30.97 g/mol). The Ca:P molar ratios investigated were ≤0.50, 0.51-0.57, 0.58-0.64 and ≥0.65, representing the 1st, 2nd, 3rd and 4th quartiles, respectively. The intake of dietary Ca and P in each Ca:P quartile is presented in Table 15.

Table 15. *Mean daily dietary energy and nutrient intakes and calcium-to-phosphorus ratio (Ca:P ratio) (±SEM) of participants (n=147) in the each quartile (Q).*

Variable	Ca:P molar ratio ≤0.50 (1st Q) (n=38)	Ca:P molar ratio 0.51-0.57 (2nd Q) (n=36)	Ca:P molar ratio 0.58-0.64 (3rd Q) (n=39)	Ca:P molar ratio ≥0.65 (4th Q) (n=34)
Energy intake (MJ/d)	7.7 (0.4)	7.5 (0.3)	8.5 (0.4)	8.0 (0.3)
Calcium intake (mg/d)	742 (41.0)	908 (40.5)	1253 (45.3)	1339 (84.6)
Phosphorus intake (mg/d)	1319 (61.9)	1299 (56.5)	1582 (54.2)	1438 (80.7)
Ca:P weight ratio (mg:mg)	0.56 (0.014)	0.70 (0.004)	0.79 (0.004)	0.92 (0.013)
Ca:P molar ratio (mol:mol)	0.42 (0.011)	0.53 (0.003)	0.60 (0.003)	0.70 (0.010)
Protein intake (g/d)	74 (4)	66 (3)	78 (3)	75 (4)
Sodium intake (g/d)	2.1 (0.1)	1.7 (0.1)	1.7 (0.1)	2.0 (0.2)

4.4 Ethical issues

The Helsinki University Ethics Committee approved the study protocols in Studies I, III and IV. In Study II, the Ethics Committee of Public Health and Epidemiology for the Hospital District of Helsinki and Uusimaa approved the study protocol. All participants gave their informed consent to the procedures, which were in accord with the Helsinki Declaration, before entering the study. Participants could withdraw their participation at any time for any reason. The results of personal nutrient intakes were explained to all participants.

4.5 Sampling

Blood collections were performed in the mornings between 7:30 and 9:15 to avoid the confounding effect of diurnal rhythm of PTH on the results (Calvo *et al.* 1991, Herfart *et al.* 1992b). In each study (I-IV), the first blood samples were taken anaerobically after a 12-h overnight fast. In the controlled studies (I, II), each study day began at 08:00, and blood samples were taken at 7:45-8:15. The samples taken and all sampling procedures of Studies I and II are presented in Fig. 7 (Study I) and Fig. 8 (Study II). In Studies III and IV, blood samples were taken between 07:30 and 09:15. In all studies, Venojet gel tubes were used to obtain clear sera. Blood samples were processed within 1 h and centrifuged at 3000 rpm for 15 min.

In Studies I and II, the 24-h urine collections were started at 08:00 in each study session, ending at 08:00 the following morning. In the cross-sectional study (III, IV), 24-h urine collections were carried out three times to obtain accurate information about the mean urinary sodium (U-Na) and U-Ca excretions of participants for the original study design (Lamberg-Allardt *et al.* 2001). On the study days, morning urine was voided and discarded at home, thus, urine collections were started from the second void urine samples.

In all of the studies, the portions of urine and separated serum samples were stored at –20 ºC until analysis. In addition, in Studies I and II two 500 µl portions of each serum sample were stored at –70 ºC.

4.6 Laboratory methods

In each study, laboratory measurements were conducted only after the sample collection was completed. All samples from the same person were analysed in the same assay in a randomized order. In addition, all samples within one study were measured at the same time and using assays with the same serial numbers to avoid variation and to assure maintenance of good laboratory practices. A summary of the laboratory methods used in

each study and intra- and inter CV% of methods are presented in Table 16. All analyses in Studies I-IV were conducted at the laboratory of the Department of Applied Chemistry and Microbiology (Division of Nutrition), University of Helsinki.

Table 16. *Laboratory measurements in Studies I-IV.*

Variable	Study	Method	CV% intra	CV% inter
Serum measurements				
Serum ionized calcium (S-iCa)	I-IV	Ion selective analyser	1.6	-
Serum intact parathyroid hormone (S-PTH)	I-IV	IRMA	1.0	<4.0
Serum bone-specific alkaline phosphatase (S-BALP)	I, II	ELISA	<5.5	<7.5
Serum 25-hydroxyvitamin D (S-25-OHD)	III, IV	RIA	10.1	14.9
Serum 1,25-dihydroxyvitamin D (S-1,25-OH_2D)	I	RIA	9.0	10.0
Serum calcium (S-Ca)	I-IV	Spectrophotometric	<2.0	<3.5
Serum phosphate (S-Pi)	I-IV	Spectrophotometric	<2.9	<3.5
Serum creatinine (S-Cr)	I-IV	Spectrophotometric	<2.0	<3.5
Urinary measurements				
Urinary calcium (U-Ca)	I, II, IV	Spectrophotometric	<2.0	<3.5
Urinary phosphate (U-Pi)	I, II, IV	Spectrophotometric	<2.9	<3.5
Urinary creatinine (U-Cr)	I, II, IV	Spectrophotometric	<2.0	<3.5
Urinary N-terminal telopeptide of collagen I (U-NTx)	I, II	ELISA	9.7	11.8
Urinary sodium (U-Na)	IV	Ion selective analyser	<3.5%	-

ELISA=enzyme-linked immunoassay; IRMA=immunoradiometric assay; RIA=radioimmunoassay

Serum ionized calcium (S-iCa) concentration was measured within 90 min of sample collection with an ion selective analyser (Microlyte 6, Thermo Electron Corp., Vantaa, Finland). In each study, serum intact PTH concentration was determined using an immunoradiometric assay (Nicholas Institute, Juan San Capistrano, CA, USA). Serum 25(OH)D concentration was measured only in Studies III and IV by a radioimmunoassay (Incstar Corp., Stillwater, MN, USA). In Study I, 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 P dose session from the 0-, 8-, 10- and 24-h samples. Serum Ca, phosphate, creatinine and urinary Ca and phosphate and creatinine concentrations were measured by routine laboratory methods using a Konelab 20 Automatic Analyser (Thermo Electron Corporation, Vantaa, Finland) in Studies I and II and an Elan Automatic Analyser (Eppendorf-Netheler-Hinz GmbH, Germany) in Studies III and IV. Urinary sodium (U-Na) concentrations were measured with an ion selective analyser (Microlyte 6, Thermo Electron Corp., Vantaa, Finland). In Study IV, the average of three days' urine collection was used to calculate U-Ca, U-Pi and U-Na.

Bone-specific alkaline phosphatase (BALP) was analysed with an enzyme immunoassay (Metra Biosystems, Palo Alto, CA, USA) in Studies I and II. The BALP analyses were performed on the 0-mg (control), 750-mg and 1500-mg P dose sessions from the 0-, 8-, 10- and 24-h samples in Study I. In Study II, BALP concentration was analysed from the 0-mg (control) and the 1200-mg Ca dose blood samples with an enzyme immunoassay (Metra ™ BAP EIA Kit, Quidel Corp., San Diego, CA, USA). The concentrations of the urinary N-terminal telopeptide of collagen I (U-NTx) were determined with an ELISA Osteomark NTx test (Ostex International Inc., Seattle, WA, USA) in Studies I and II. In Study II, U-NTx was measured from all urine samples, while in Study I, U-NTx was measured only from the 0-mg (control), 750-mg and 1500-mg P dose sessions.

4.7 Statistical methods

The data are expressed as means±SEM. The variables were tested for normality, and logarithmic transformations were used to normalize non-normal distributions. SPSS software program version 10.0 (SPSS Inc., Chicago, IL, USA) was used in Studies I and II, version 12.07 in Study III and version 15.0 in Study IV. All versions of SPSS software were used in a Windows environment for all statistical analyses. Results were considered statistically significant when $p < 0.05$. In tables, the standard error of the mean (SEM) is presented in parentheses after the mean value. In figures, values are means with their SEM indicated by vertical bars.

4.7.1 Controlled studies

In Studies I and II, ANOVA with repeated measures was used to compare the study periods. If the spherity assumption was violated, Hyunh-Feldt adjustment was used. The effects of the P (Study I) and Ca (Study II) doses were compared with the control session with contrast analysis. In Study I, for serum variables the area under the curve (AUC) for differences from the morning fasting value was calculated.

4.7.2 Cross-sectional studies

In Study III, the associations of dietary phosphate additives and natural P with the central markers of Ca and bone metabolism were investigated with a statistical approach originating from an elaboration technique (Babbie 2001). First, the associations of phosphate additives or natural P were estimated by comparing means of the variables measured (e.g. S-PTH) among the groups. Next, potential distortions of these averages were removed by adjusting for the effects of relevant covariates by using analysis of covariance (ANCOVA). Finally, the two averages were adjusted for total P intake to exclude the effect of increased total P intake. The critical point with respect to testing the

hypothesis was whether the difference between the groups prevails even after adjustment; if so, the hypothesis gains support.

To examine the associations of total P intakes (Study III) and Ca:P ratios (Study IV) with Ca and bone metabolism, the differences in the averages of the dependent variables between quartiles were compared with ANCOVA. ANCOVA was used because it enables inclusion of both categorical (e.g. use of contraceptives) and continuous (e.g. S-iCa) explanatory variables. In Study IV, analysis of variance (ANOVA) was also used to compare differences in the averages of the dependent variable between Ca:P quartiles. After ANOVA, ANCOVA was used to exclude the possibility that differences found with ANOVA were due to differences in relevant covariates. For pairwise comparison when ANOVA or ANCOVA p-values were <0.05, Fisher`s least significant difference (LSD) method was used.

In Studies III and IV, covariates that correlated with outcome parameter or covariates known to affect outcome according to the literature were chosen. Age was excluded from the covariates, as all participants represented the same age group and all were premenopausal women. Energy intake was also excluded because Ca and P intakes correlated well with energy intake. In addition, body weight and body mass index (BMI) had no effects on the variables measured, and thus, were not included in the covariates.

Several factors are known to affect S-PTH concentration. Among these are nutrient intakes (Ca, P, Na) and serum variables (S-25(OH)D and S-iCa). The use of hormonal contraceptives might also influence S-PTH, as when compared with non-users hormonal contraceptive users had lower S-PTH concentrations (Teegarden *et al*. 2005). In Study III, when we examined the associations of total P intake with S-PTH, dietary Ca and sodium (Na) intakes, S-25(OH)D and S-iCa were used as covariates. When the associations of P from natural P and phosphate additives with S-PTH were investigated, total P intake was used in the final stage to adjust the two averages for total P intake to exclude the effects of increased total P intake due to consumed processed cheese or higher milk and cheese consumption. When the means of S-iCa were compared, S-PTH was included and Na intake excluded from covariates.

In Study IV, when the means of S-PTH were compared, S-25(OH)D and S-iCa concentrations and the use of contraceptives were included in covariates. When the means of S-iCa, S-Ca and S-Pi were compared, S-PTH was included in covariates. In addition, S-Pi was included in covariates when comparing the means of S-iCa and S-Ca. Dietary protein intake has been demonstrated to have hypercalciuric effects (Hegsted *et al.* 1981, Lakshmanan *et al.* 1984, Kerstetter *et al.* 2003), and dietary Na intake (Nordin *et al.* 1993, Evans *et al.* 1997) affects U-Ca excretion; therefore, when the means of U-Ca were compared, dietary Na and protein intakes were included in covariates.

5 Results

5.1 Habitual dietary phosphorus and calcium intakes and dietary calcium-to-phosphorus ratio (Studies I-IV)

As presented in Table 17, habitual dietary phosphorus (P) intake of the participants was high in each study. For 42-93% of participants, dietary P intake exceeded over 2-fold the dietary recommendations for P (600 mg/d) (National Nutrition Council 2005).

Table 17. *Habitual dietary phosphorus intake of participants in Studies I-IV.*

Study	Mean P intake (mg/d)	Range of P intake (mg/d)	P intake >1200 mg/d (% of participants)*	Mean energy intake (MJ/d) (SEM)
I	1501 **	1006-2022	93	7.9 (0.5)
II	1247 **	927-1734	42	7.7 (0.2)
III, IV	1411 **	601-3010	67	7.9 (0.2)

* 2-fold higher than recommended intake for P (600 mg/d) in Finland (National Nutrition Council 2005)
** Corresponds with an average intake of P in Finnish females (1363 mg/d, SD 448) (Paturi *et al*. 2008)

The mean habitual dietary calcium (Ca) intake of the participants was adequate in each study (Table 18). However, some participants had a Ca intake below recommended levels (800 mg/d) (National Nutrition Council 2005), although the mean Ca intake among these individuals was mostly not much below the recommendations (Table 18).

Table 18. *Habitual dietary calcium intake of participants in Studies I-IV.*

Study	Mean Ca intake (mg/d)	Range of Ca intake (mg/d)	Ca intake <800 mg/d, % of participants (mean Ca intake among these participants, mg/d)*	Mean energy intake (MJ/d) (SEM)
I	1134**	575-1908	21 (706)	7.9 (0.5)
II	883**	405-1501	42 (634)	7.7 (0.2)
III, IV	1056**	200-2739	30 (647)	7.9 (0.2)

* Recommended Ca intake in Finland 800 mg/d (National Nutrition Council 2005)
** Corresponds to the average Ca intake in Finnish females (1007 mg/d, SD 450) (Paturi *et al.* 2008)

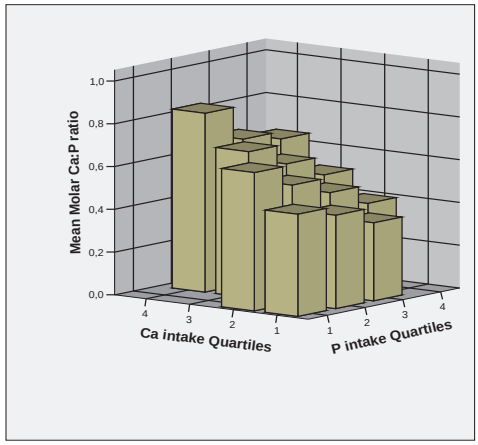
Both the weight and the molar calcium-to-phosphorus ratios (Ca:P ratio) of participants in Studies I-IV are presented in Table 19. Molar dietary Ca:P ratio varied between 0.22 and 0.91. The mean molar Ca:P ratios of Study IV participants in each quartile of dietary Ca and P intake are presented in Figure 9. As shown in Figure 9, there were three groups with no subjects: the group with the lowest Ca intake (<770 mg/d) and the highest P intake (>1643 mg/d), the group with the second highest Ca intake 998-1251 mg/d and the lowest P intake (<1122 mg/d) and the group with the highest Ca intake (>1251 mg/d) and the lowest P intake (<1122 mg/d). For all participants in Study IV, the Ca:P molar ratio at the

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

Table 19. *Habitual dietary Ca:P ratios of participants in Studies I-IV.*

Study	Mean Ca:P weight ratio (mg:mg)*	Range of Ca:P weight ratios	Mean Ca:P molar ratio (mol:mol)*	Range of Ca:P molar ratios
I	0.74**	0.55-0.94	0.57	0.42-0.72
II	0.70**	0.37-0.87	0.54	0.28-0.67
III, IV	0.74**	0.29-1.18	0.57	0.22-0.91

* Based on calculations of recommended dietary Ca and P intakes, the optimal dietary Ca:P molar ratio is suggested to be 1 (e.g. SCF 1993, Calvo and Park 1996), corresponding to a Ca:P weight ratio of 1.3.
* *Corresponds to the average Ca:P weight ratio of Finnish females (Ca:P ratio: 0.74) (Paturi *et al*. 2008).



Dietary Ca intake in Ca quartiles:
1st: <770 mg/d
2nd: 771-997 mg/d
3rd: 998-1251 mg/d
4th: >1251 mg/d

Dietary P intake in P quartiles:
1st: <1122 mg/d
2nd: 1122-1347 mg/d
3rd: 1348-1643 mg/d
4th: >1643 mg/d

Figure 9. *Mean molar dietary Ca:P ratios (y-axis) in each Ca (x-axis) and P (z-axis) intake quartile.*

5.2 Associations of dietary phosphorus doses and sources with calcium and bone metabolism (Studies I and III)

5.2.1 Acute effects of four different phosphorus doses (Study I)

Oral intake of different P doses (0, 250, 750 and 1500 mg) with low Ca intake (250 mg/d) acutely increased serum phosphate (S-Pi) and serum parathyroid hormone (S-PTH) concentrations and urinary phosphate (U-Pi) excretion in a dose-dependent manner. Furthermore, the highest P dose (1500 mg) decreased serum ionized calcium (S-iCa) concentration and bone formation, increased bone resorption and inhibited the increase in serum $1,25(OH)_2D$ (S-$1,25(OH)_2D$) concentration in response to low dietary Ca intake. These results are presented in greater detail below.

5.2.1.1 Serum Pi and ionized Ca concentrations

A significant dose-response relationship was observed in the S-Pi concentration in relation to P doses ($p<0.001$, ANOVA; Fig. 10, panel A). A significant increase was already found with the 250-mg P dose ($p=0.04$, contrast analysis), and the increase was more profound after the 750-mg ($p=0.002$, contrast analysis) and 1500-mg ($p<0.001$, contrast analysis) P doses. In 4 of the 14 subjects, the S-Pi level increased above the normal reference limit (>1.4 mmol/l) with the 750-mg P dose, and in 7 of the 14 subjects with the 1500-mg P dose. S-iCa concentration declined (Fig. 10, panel B) in response to P intake ($p<0.001$, ANOVA), but was significant only after the 1500-mg P dose ($p=0.004$, contrast analysis). In addition, S-Pi concentration tended to be higher ($p=0.09$) and S-iCa was still diminished ($p=0.004$) in the morning following the 1500-mg P dose than in the previous-day morning fasting sample (Fig. 10).

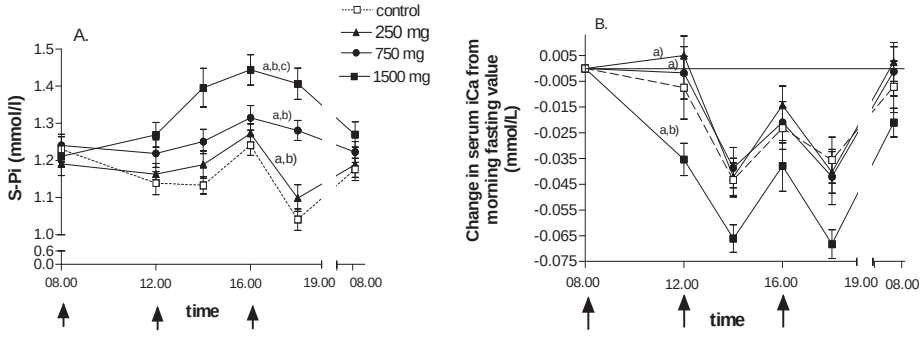


Figure 10. *Change in serum phosphate (S-Pi) (panel A) and serum ionized calcium (S-iCa) (panel B) concentrations during study days. SEM of mean values is depicted by vertical bars. The arrow indicates P administration time.* $^{a)}p< 0.05$ *by ANOVA, repeated measures design. Significantly different* $^{b)}p< 0.05$ *and* $^{c)}p< 0.001$ *from control day by contrast analysis.*

5.2.1.2 Serum PTH concentration

S-PTH concentration increased in a dose-dependent manner in response to P intake ($p<0.001$, ANOVA; Fig. 11). Contrast analysis showed that these increments were significant at all doses ($p=0.03$, $p=0.002$ and $p<0.001$ at 250-, 750- and 1500-mg P doses, respectively). The maximum difference in S-PTH from the morning fasting level occured 2 h after the last dose of P (at 18:00), being 20% ($p=0.3$, 250-mg P dose), 32% ($p=0.009$, 750-mg dose) and 57% ($p=0.006$, 1500-mg dose) above the morning fasting value. With the two highest P doses, these increases were significant ($p=0.03$ and $p<0.001$, respectively, contrast analysis) compared with the 12% increment during the control session. Of the 14 subjects, three during the 1500-mg P dose session and one during the 750-mg session had S-PTH values above the upper reference limit (> 65 ng/l). In addition, the diurnal variation of the S-PTH concentration varied between the two highest P doses

and control and the 250-mg P dose. Between the time-points at 12:00 and at 14:00, S-PTH concentrations increased with the 750-mg and 1500-mg P doses, while a slight decrease occurred in the S-PTH concentrations with both the control and the 250-mg P dose.

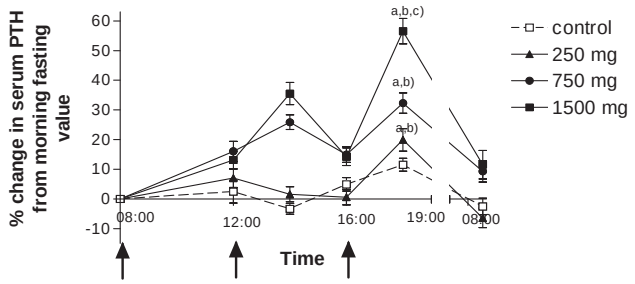


Figure 11. *Change in parathyroid hormone (PTH) concentration during study day. SEM of mean values is depicted by vertical bars. The arrow indicates P administration times.* [a]*p< 0.05 by ANOVA, repeated measures design. Significantly different* [b]*p< 0.05 and* [c]*p< 0.001 from control day by contrast analysis.*

5.2.1.3 Bone formation and resorption markers

There was a decline in a marker of bone formation, serum bone alkaline phosphatase (S-BALP) activity, after the 750-mg and 1500-mg P doses (p=0.009, ANOVA) (Fig. 12, panel A). The 1500-mg P dose decreased S-BALP activity significantly (p=0.004, contrast analysis), but the 750-mg dose caused no significant decline (p=0.75, contrast analysis) compared with the control session. The bone resorption marker, the 24-h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion (U-NTx/U-Cr) (Fig. 12, panel B), was affected by P intake (p=0.048, ANOVA). With the 1500-mg P dose, U-NTx/U-Cr was 33% (p=0.06, contrast analysis) above the level of the control day.

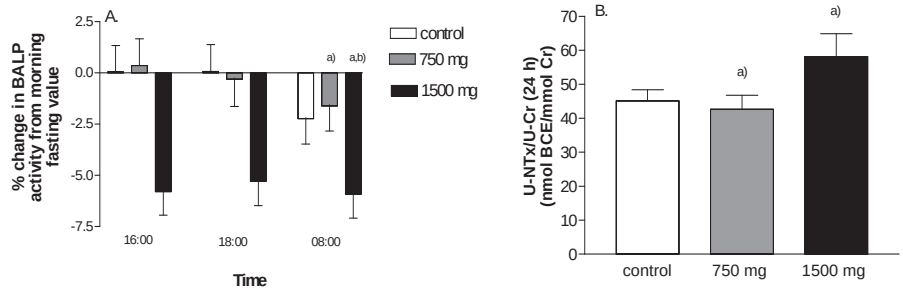


Figure 12. *Change in serum bone-specific alkaline phosphatase (BALP) activity during study days in panel A. The 24-hour urinary excretion of NTx/Cr during study days is shown in panel B. Values are presented with SEM of mean values.* [a]*p < 0.05 by ANOVA, repeated measures design. Significantly different* [b] *p< 0.05 from control day by contrast analysis.*

5.2.1.4 Serum 1,25(OH)$_2$D concentration

The serum calcitriol (S-1,25(OH)$_2$D) concentration did not change significantly in response to the 1500-mg P dose (p=0.2, ANOVA, Fig. 13). However, the S-1,25(OH)$_2$D concentration had increased on the following morning (24 h) at the control session compared with the previous morning fasting value (p=0.05), whereas at the 1500-mg P dose no change occured (p=0.9).

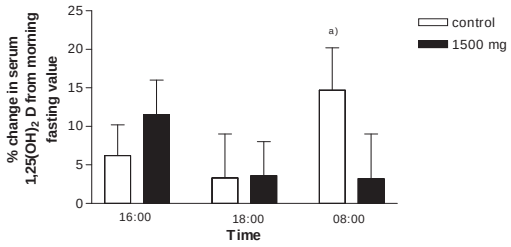


Figure 13. *Change in serum 1,25-dihydroxyvitamin D during study days. Values are presented with SEM of mean values. Significantly different a) P< 0.05 from morning fasting value of the control day.*

5.2.1.5 24-h urinary Pi and Ca excretions

The urinary phosphate (U-Pi) excretion (Fig. 14, panel A) increased in a dose-dependent manner (p<0.001, ANOVA) with increasing P doses. The U-Pi excretion increased by 27% with the 250-mg P dose (p=0.08, contrast analysis), 70% with the 750-mg dose (p<0.001, contrast analysis) and 126% with the 1500-mg dose (p<0.001, contrast analysis). In addition, the 24-h urinary calcium (U-Ca) excretion (Fig. 14, panel B) decreased significantly with increasing P doses (p<0.001, ANOVA). The P doses of 750 mg and 1500 mg both decreased the U-Ca excretion significantly (p<0.001 and p=0.002, respectively, contrast analysis) and in a similar manner compared with the control session.

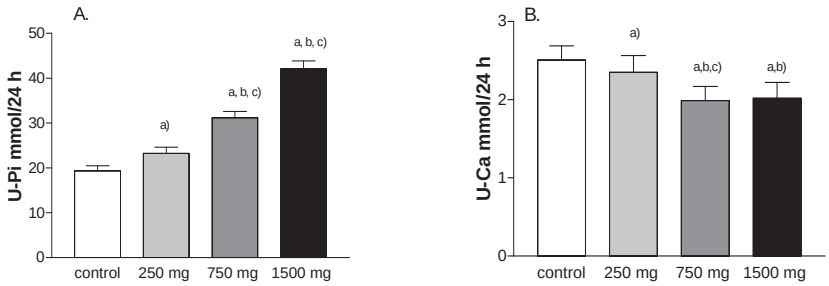


Figure 14. *24-h urinary phosphate (A) and calcium (B) excretion during study days. Values are means with SEM. a) p<0.05 by ANOVA, repeated measures design. Mean values are significantly different from those of the control day by contrast analysis: b)p<0.05, c)p<0.001.*

5.2.2 Associations of habitual dietary phosphorus intakes with serum parathyroid hormone concentration and calcium metabolism (Study III)

In a cross-sectional study, a high habitual dietary P intake (>1648 mg/d) was associated with lower S-iCa and higher S-PTH concentrations. The mean total P intake was 961 (SEM 21.9) mg/d in the 1st quartile and 1956 (SEM 47.8) mg/d in the 4th quartile. These results are presented in greater detail below.

5.2.2.1 Serum ionized Ca and PTH concentrations

Compared with the 1st quartile, the mean S-iCa concentration was lower in the 4th quartile (p=0.016, ANCOVA) after adjustment for dietary Ca intake, S-PTH and 25(OH)D concentrations and use of hormonal contraceptives (Fig. 15, panel A). Relative to the 1st quartile, the mean S-PTH concentration was 1.8-fold higher in the 4th quartile (S-PTH (ng/l): 21.7 and 39.4 for the 1st and 4th quartile, respectively; p=0.048, ANCOVA) after adjustments for serum 25-hydroxyvitamin D and S-iCa concentrations, total dietary Ca and Na intakes and use of contraceptives (Fig. 15, panel B).

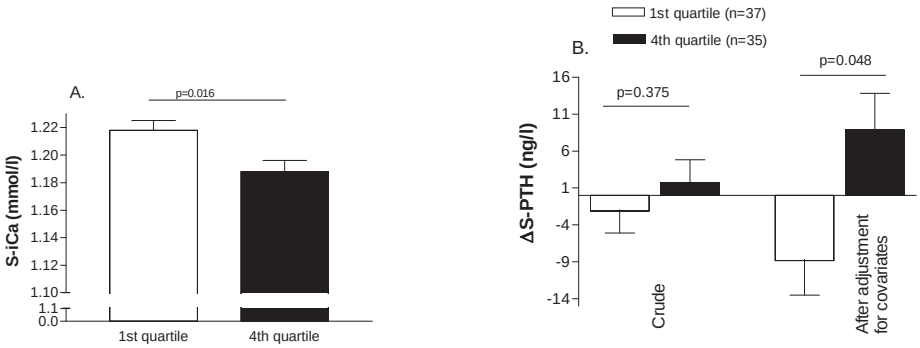


Figure 15. *Associations of total dietary phosphorus with serum ionized calcium (S-iCa) (panel A) and parathyroid hormone (S-PTH) (panel B) concentrations (mean ±SEM) in the 1st and 4th quartiles of total P intake. ΔS-PTH values were calculated from the mean S-PTH concentration (30.6 ng/l). Analysis of covariance (ANCOVA) was performed.*

5.2.3 Associations of dietary phosphorus sources with serum parathyroid hormone concentration and calcium metabolism (Study III)

In the habitual diets, the consumption of foods with phosphate additives (processed cheese) was associated with higher mean S-PTH concentrations, whereas consumption of foods with natural P (milk and cheese, excluded processed cheese) was associated with lower mean S-PTH concentrations. These results are presented in more detail below.

5.2.3.1 Serum ionized Ca and PTH concentrations

The mean S-iCa concentration did not differ between consumers and non-consumers of processed cheese (p=0.9, ANCOVA) or between groups of low and high milk and cheese consumption (p=0.7, ANCOVA) after adjustment for dietary P and Ca intakes, S-PTH and S-25(OH)D concentrations and use of contraceptives.

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=0.19, ANCOVA), probably due to different group sizes and the significant interaction (p=0.023, ANCOVA) found between processed cheese consumption and use of hormonal contraceptives. Therefore, the associations of processed cheese with S-PTH were evaluated in the subgroups of hormonal contraceptive users (n=39) and non-users (n=108). As shown in Fig. 16, panel A, among hormonal contraceptive users, mean S-PTH did not differ significantly between consumers and non-consumers of processed cheese even after adjusting for four covariates and total P intake (p=0.15, ANCOVA). Among those who did not use contraceptives, S-PTH was higher with processed cheese consumption (S-PTH (ng/l): 36.5 and 29.5 for consumers and non-consumers, respectively) after adjustment for four covariates and total P intake (p=0.027, ANCOVA) (Fig. 16, panel B).

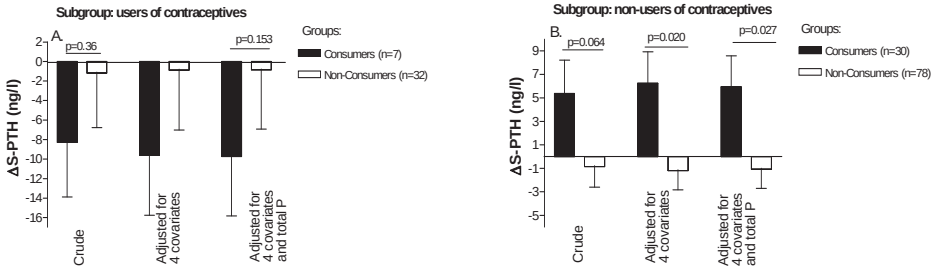


Figure 16. *Associations of processed cheese consumption with serum parathyroid hormone (S-PTH) concentration in consumers and non-consumers of processed cheese. The associations were studied in subgroups of hormonal contraceptive users (panel A) and non-users (panel B). ΔS-PTH values were calculated from the mean S-PTH concentration (30.6 ng/l). The four covariates used in the model included serum 25-hydroxyvitamin D and ionized Ca concentrations and total dietary Ca and Na intakes. At the final stage, total dietary P intake was added to the model. Analysis of covariance was performed.*

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 total P intake. As a significant interaction between S-iCa concentration and consumption of milk and cheese (p=0.009, ANCOVA) was found, the association of milk and cheese with S-PTH concentration were examined in the subgroups of S-iCa <1.225 mmol/l (n=114) and S-iCa ≥1.225 mmol/l (n=33). The S-iCa concentration 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. 17, panel A). By contrast, at 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=0.36, ANCOVA) after adjustment for the four covariates and total P intake (Fig. 17, panel B).

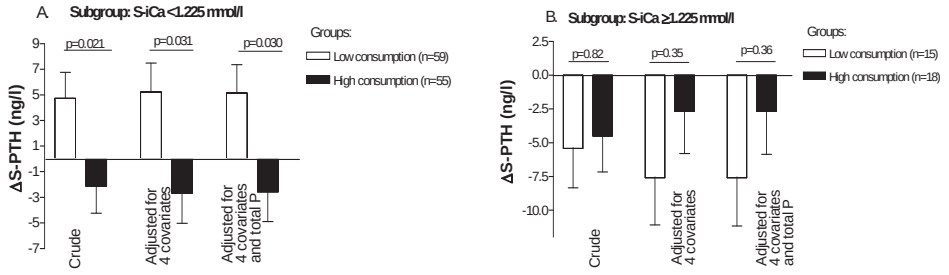


Figure 17. *Associations of milk and cheese consumption with serum parathyroid hormone (S-PTH) concentration in groups of low and high milk and cheese consumption. The associations were studied in the subgroups of those whose serum ionized calcium concentration was <1.225 mmol/l (panel A) and those whose concentration was ≥1.225 mmol/l (panel B). ΔS-PTH values were calculated from the mean S-PTH concentration (30.6 ng/l). The four covariates used in the model included serum 25-hydroxyvitamin D and ionized Ca concentrations and total dietary Ca and Na intakes. At the final stage, total dietary P intake was added to the model. Analysis of covariance was performed.*

5.3 Acute effects of increasing calcium intakes on calcium and bone metabolisms when dietary phosphorus intake is high (Study II)

Acutely, when dietary P intake was 3-fold above the dietary guidelines (1850 mg/d), by increasing the oral Ca intake from 480 mg/d (control day) to 1080 mg/d (600-mg Ca dose) and 1680 mg/d (1200-mg Ca dose), S-iCa concentration increased, S-PTH concentration decreased and bone resorption decreased dose-dependently. In addition, U-Ca excretion increased and U-Pi excretion decreased in a dose-dependent manner. Ca doses did not affect bone formation. The results of Study II are presented in greater detail below.

5.3.1 Serum Pi, ionized Ca and PTH concentrations

No significant change was observed in S-Pi concentration due to increasing Ca doses (p=0.6, ANOVA). However, S-Pi increased above the normal reference limit (>1.4

mmol/l) in 8 of the 12 subjects on the control day, in 6 of the 12 subjects on the 600-mg Ca dose day and in 4 of the 12 subjects on the 1200-mg Ca dose day.

A significant dose-response relationship was observed between S-iCa concentration and Ca intake ($p<0.001$, ANOVA) (Fig. 18, panel A). Compared with the control day, the 1200-mg Ca dose increased S-iCa ($p=0.001$, contrast analysis) more efficiently than did the 600-mg dose ($p=0.02$, contrast analysis). In addition, the S-iCa concentration dropped 4 h after commencement of the study on the control day, while the concentration remained nearly constant on both the 600-mg and 1200-mg Ca days. The S-PTH concentration decreased in a dose-dependent manner in response to the Ca doses ($p<0.0005$, ANOVA) (Fig. 18, panel B). Contrast analysis revealed that the increase was equally significant on the 600-mg and 1200-mg Ca days ($p=0.001$). On the control day, when the Ca intake was low, 6 of the 12 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 600-mg and the 1200-mg Ca dose days between 14:00 and 19:00. While the concentration almost returned to the baseline value at 19:00 in the 600-mg and 1200-mg dose sessions, the S-PTH value continued to increase on the control day.

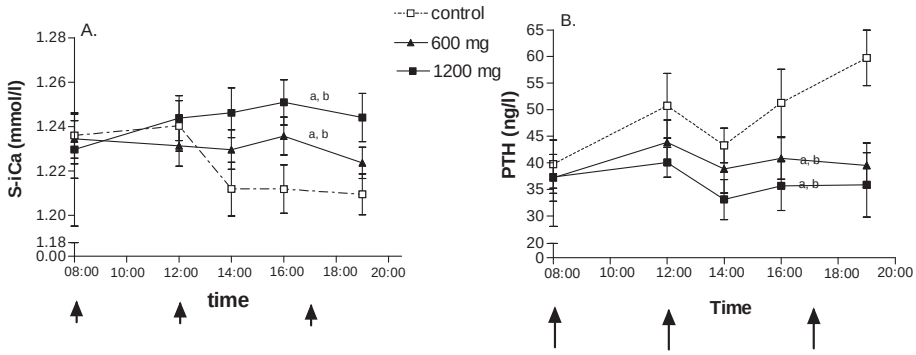


Figure 18. *Change in serum ionized calcium (panel A) and serum parathyroid hormone (panel B) concentration during experiment days. Values are means with their SEM depicted by vertical bars.↑: Ca administration times.* $^{a)}P<0.05$ *by ANOVA, repeated measure design.* $^{b)}P<0.05$*: mean values were significantly different from those of the control day by contrast analysis.*

5.3.2 Bone formation and resorption markers

The bone formation marker S-BALP did not differ significantly from the control day ($p=0.4$, ANOVA, Fig. 19, panel A). However, Ca doses affected the excretion of a bone resorption marker, the 24-h U-NTx/U-Cr ($p=0.008$, ANOVA) (Fig. 19, panel B). The excretion of U-NTx diminished significantly with both Ca doses ($p=0.009$ and $p=0.02$ for 600-mg and 1200-mg Ca doses, respectively, contrast analysis), being 14% lower on the 600-mg dose day and 17% lower on the 1200-mg dose day than on the control day.

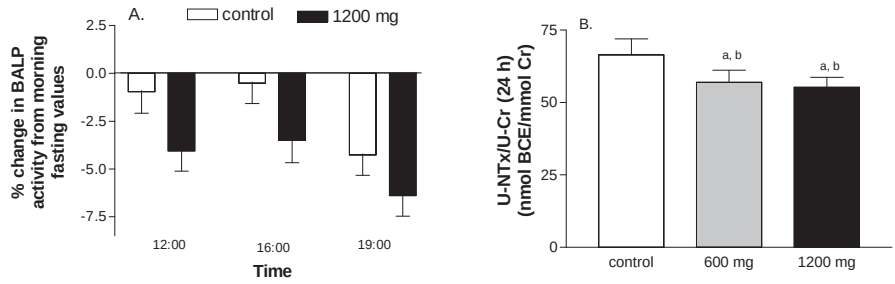


Figure 19. *Change in serum bone-spesific alkaline phosphatase (BALP) activity from morning fasting values and the mean values of 24-h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion (NTx:Cr) (panel B) during experiment days. Values are presented with SEM of mean values.* $^{a}P<0.05$ *by ANOVA, repeated measure design.* $^{b}P<0.05$*: mean values were significantly different from those of the control day by contrast analysis.*

5.3.3 24-h urinary Pi and Ca excretions

The 24-h U-Pi decreased (p=0.005, ANOVA) (Fig. 20, panel A) and the 24-h U-Ca increased (p=0.004, ANOVA) (Fig. 20, panel B) with increasing Ca doses. The 1200-mg Ca dose decreased U-Pi more efficiently (p=0.002, contrast analysis) than the 600-mg dose (p=0.089, contrast analysis). By contrast, the 1200-mg Ca dose increased U-Ca by 58% (p=0.001, contrast analysis), while the 600-mg dose increased U-Ca less (38%) (p=0.12, contrast analysis).

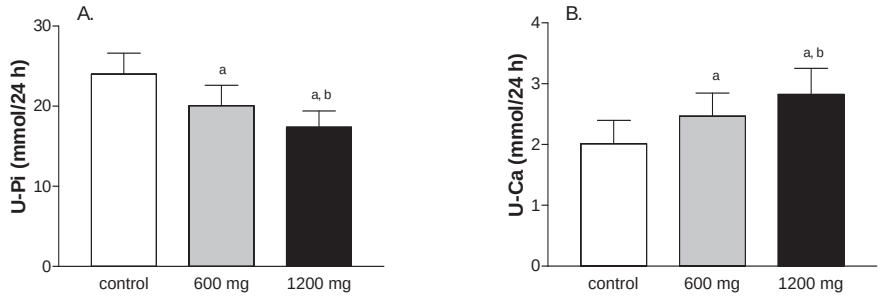


Figure 20. *24-h urinary phosphate (panel A) and urinary calcium (panel B) excretion during study days. Values are means with SEM.*$^{a)}p<0.05$ *by ANOVA, repeated measure design.* $^{b)}p<0.05$*; mean values were significantly different from those of the control day by contrast analysis.*

5.4 Associations between habitual dietary calcium-to-phosphorus ratios and serum parathyroid hormone concentration and calcium metabolism (Study IV)

Habitual low dietary Ca:P ratios (Ca:P molar ratios ≤0.50) were associated with higher S-PTH concentrations and 24-h urinary Ca excretions than higher Ca:P ratios in Study IV. The 1st Ca:P ratio quartile (Ca:P molar ratio ≤0.50) differed from all other quartiles in its effects on S-PTH and U-Ca. Quartiles other than the 1st one were similarly associated with the markers measured. These results are presented in more detail below.

5.4.1 Serum PTH concentration

The mean S-PTH concentration differed significantly between the Ca:P quartiles (p=0.014, ANOVA), being higher in the 1st quartile than in the others. To exclude the possibility that these differences are not due to differences in relevant covariates, the S-PTH means were adjusted for the following variables: S-iCa and S-25-OH-D concentrations and use of contraceptives. After these adjustments, the S-PTH differences remained practically the same, as did their statistical significance (p=0.021, ANCOVA) (Fig. 21, panel A). The mean S-PTH concentration was 30% higher in the 1st quartile than in the combined group of the 2nd, 3rd and 4th quartiles (p=0.002) (Fig. 21, panel B). No differences in the mean S-PTH concentrations were present among the 2nd, 3rd and 4th quartiles, and the smallest p-value among quartiles in the ANCOVA model was only p=0.88.

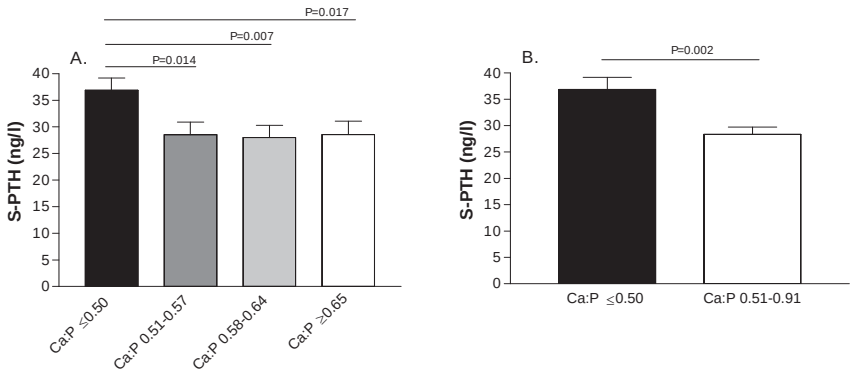


Figure 21. *Associations of calcium-to-phosphorus ratios (Ca:P) with serum parathyroid hormone (S-PTH) concentration (mean±SEM) in the different quartiles (panel A) and in the 1st quartile (n=38) and in the combined group of the 2nd, 3rd and 4th quartiles (n=109) (panel B). Values are means with SEM. Analysis of covariance (ANCOVA) was performed. P-values presented in panel A are the mean values different from those of the 1st quartile (Fisher`s LSD comparison, ANCOVA).*

5.4.2 24-h urinary Ca excretion

Dietary Ca:P ratio was significantly associated with the 24-h U-Ca excretion (p=0.047, ANOVA). The mean U-Ca excretion in the 1st quartile was higher than in the other quartiles. After adjusting the U-Ca means for the relevant covariates (S-25OH-D, S-PTH, dietary Na and protein intakes, use of contraceptives), similar associations and statistical significance were still noted (p=0.051, ANCOVA) (Fig. 22, panel A). When comparing the 2nd, 3rd and 4th quartiles as one group to the 1st quartile, the mean U-Ca excretion was around 30% higher in the 1st quartile (p=0.006, ANCOVA) (Fig. 22, panel B). The mean U-Ca excretion did not vary between the 2nd, 3rd and 4th quartiles (ANCOVA; p=0.58-0.90).

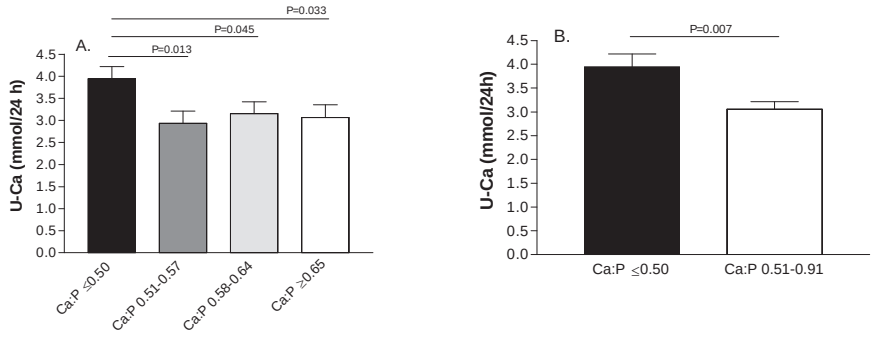


Figure 22. *Association of calcium-to-phosphorus ratios (Ca:P) with urinary calcium excretion (U-Ca) (mean±SEM) in the 1st, 2nd, 3rd and 4th quartiles (panel A) and in the 1st quartile (n=38) and in the combined group of the 2nd, 3rd and 4th quartiles (n=109) (panel B). Values are means with SEM. Analysis of covariance (ANCOVA) was performed. P-values presented in the panel A are the mean values different from those of the 1st quartile (Fisher`s LSD comparison, ANCOVA).*

6 Discussion

6.1 Effects of dietary phosphorus intakes and sources on calcium and bone metabolism

6.1.1 Dietary phosphorus intakes

Phosphorus (P) intakes higher than the dietary guidelines (>600 mg/d) (National Nutrition Council 2005) in a dose-dependent manner negatively affected calcium (Ca) and bone metabolism in a 24-h controlled study (I). The higher the P intake, the more negative the effects. In line with the observations in the controlled study, findings from the cross-sectional study (III) showed negative associations between high habitual dietary P intakes and serum parathyroid hormone (S-PTH) and ionized calcium (S-iCa) concentrations. In both studies, the effects of dietary Ca intake were ruled out. In the cross-sectional study, higher total P intake was associated with higher S-PTH and lower S-iCa concentrations even after total dietary Ca intake was equalized. In the controlled study, the intake of dietary Ca was kept low (250 mg/d) in the study sessions, as oral Ca intake also affects PTH secretion, in the direction opposite to that of dietary P. The effects of dietary P doses and habitual intakes in Studies I and III are discussed in greater detail below.

6.1.1.1 Serum PTH concentration and other calcium metabolism markers

High dietary P intakes elevated S-PTH concentration in the controlled study (I) and were associated with higher S-PTH concentrations in the cross-sectional study (III). P had a clear dose-response effects on S-PTH concentration in the controlled study; the higher the P dose, the greater the S-PTH level. Findings from earlier intervention studies with only a single high-P, low-Ca diet (Calvo *et al*. 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996) (Table 20) are in accord with the results of Studies I and III. In a controlled study by Kärkkäinen and Lamberg-Allardt (1996), a 1500-mg P dose ingested as a single dose or in three separate doses increased S-PTH secretion in healthy females. This P load corresponds to the highest P dose in Study I. Only two previous studies (Brixen *et al.* 1992, Whybro *et al.* 1998) have investigated the dose-response effect of oral P loads in humans. Unlike in Study I, in these studies dietary Ca intake was adequate (Whybro *et al.* 1998) (Table 20) or unknown (Brixen *et al.* 1992). P supplementation (1500 and 2250 mg/d) increased S-PTH concentration significantly, although P had no clear dose-response effects on S-PTH in post-menopausal women (Brixen *et al.* 1992). The unknown dietary Ca intake may explain the unclear dose-response effects. In young men, escalating P supplementation did not affect S-PTH in a dose-dependent manner during 4-week intervention periods (Whybro *et al.* 1998) (Table 20). This was probably due to timing of blood sampling and adequate dietary Ca intake (1000 mg/d), although the

P intake of 1800 mg/d did increase S-PTH by 26% compared with the P intake of 800 mg/d. The effects of P have been observed to be greater in women than in men (Calvo *et al.* 1988), which might further explain the results of Whybro *et al.* (1998).

Table 20. *Results from previous studies investigating the effects of dietary phosphorus.*

Study	Ca intake (mg/d)	P intake (mg/d)	S-iCa *	S-Pi *	S-PTH *	U-Ca *	U-Pi *
Calvo *et al.* 1988	420	1660	↓	↑	↑	↓	↑
Calvo *et al.* 1990	400	1700	↓	-	↑	↓	↑
Grimm *et al.* 2001	1995	3008	-	-	↑ NS	NR	NR
Kärkkäinen and Lamberg-Allardt 1996	375	2378 (P dose:1500)	↓	↑	↑	↓	↑
Whybro *et al.* 1998	800	800, 1800	- **	-	↑	↓	↑
Whybro *et al.* 1998	1000	1000, 2000, 2500, 3000	- **	-	-	↓	↑

*S-iCa, serum ionized calcium; S-Pi, serum phosphate; S-PTH, serum parathyroid hormone; U-Ca, urinary calcium excretion; U-Pi, urinary phosphate excretion
↓= decrease, ↑=increase, - = no effect
NS= not statistically significant, NR= not reported
**= serum calcium (S-Ca)

In several animal studies, high-P diets have caused secondary hyperparathyroidism and resulted in lower bone mineral density (BMD), especially when dietary Ca intake has been inadequate (e.g. Katsumata *et al.* 2005, Huttunen *et al.* 2006). The results from the controlled study (I) suggest that foods with high P content may cause transient secondary hyperparathyroidism also in healthy individuals, at least when dietary Ca intake is low; with the 1500-mg P dose 3 of 14 subjects and with the 750-mg dose one of 14 subjects had S-PTH values above the higher reference limit (>65 ng/l). S-PTH concentration increased already after the first ingested P load (4-h sample) with 750-mg and 1500-mg P dose sessions, indicating more harmful effects of higher P doses on S-PTH. Previous acute (Kärkkäinen and Lamberg-Allardt 1996), 8-day (Calvo *et al.* 1988) and 4-week intervention (Calvo *et al.* 1990) studies also suggested the possibility of secondary hyperparathyroidism in healthy subjects due to high-P, low-Ca diets. The P doses (250, 750 and 1500 mg) ingested in Study I were in the form of phosphate additives; thus, the effects might have been more powerful than had natural P been ingested. Phosphorus was administered as phosphate additives also in study by Kärkkäinen and Lamberg-Allardt (1996), while in Calvo *et al.* (1988, 1990) P was derived from common foods containing both natural P and phosphate additives. However, habitual higher dietary P (Study III), mainly derived from natural P, had a stronger negative association than lower intake with S-PTH in women with a generally adequate dietary Ca intake; the mean S-PTH was almost 2-fold higher among participants whose habitual P intake was >1649 mg/d compared with those whose intake was <1123 mg/d.

Phosphorus can affect S-PTH by decreasing S-iCa concentration (Reiss *et al.* 1970). In the present studies, S-iCa concentration decreased in response to high P intake in the acute situation with the 1500-mg P dose (Study I) and was associated with lower S-iCa concentrations in habitual diets with dietary P intakes >1649 mg/d (Study III). The reason why S-iCa did not decline in response to the smaller P doses in the controlled study is unclear, as the smaller doses of P (250 and 750 mg) increased S-PTH. Therefore, P might have directly affected S-PTH. However, it is unlikely that the low Ca intake would have been responsible for these low S-iCa values in either the controlled study (I) or the cross-sectional study (III); the habitual Ca intake was in general adequate or high in Study III, and the dietary Ca intake was low (250 mg/d) throughout the experiment in Study I. In addition, even a Ca load as small as 250 mg has been demonstrated to increase S-iCa and decrease S-PTH concentrations (Kärkkäinen *et al.* 2001). Unlike in Study I, Kärkkäinen and Lamberg-Allardt (1996) found no effects on S-iCa when subjects received the 1500-mg P load in three separate doses. When the 1500-mg P load was given in one dose, S-iCa concentration decreased significantly. In other studies, high P intake has been observed to decrease S-iCa in both sexes (Portale *et al*. 1987, Calvo *et al.* 1988, Calvo *et al.* 1990) or to have no effects (Calvo and Heath III 1988). In studies without a proper control session, serum total Ca (S-Ca) or S-iCa decreased (Reiss *et al.* 1970, Silverberg *et al.* 1986) or no change occurred (Renier *et al.* 1992) when comparing morning fasting values. Interestingly, S-iCa concentrations decreased in response to higher P (1660 mg/d) intake only in female participants (Calvo *et al.* 1988).

Serum calcitriol (S-1,25$(OH)_2$D) concentration increases in response to a decrease in Ca intake (Dawson-Hughes *et al.* 1993), a decrease in S-Ca and high concentrations of S-PTH (Boden and Kaplan 1990) or high dietary P intake (Portale *et al.* 1989). In the controlled study (I), an increase in S-1,25$(OH)_2$D concentration was noted after 24 h on the control day, when both the Ca and P intakes were low, as expected. However, a high P dose (1500 mg) did not change S-1,25$(OH)_2$D concentration after 24 h despite a low Ca intake and an increase in S-PTH. Our 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 Grimm *et al.* (2001), the Ca intake was high, thus counteracting the stimulus of PTH on 1-α-hydroxylase. In an 8-day study by Calvo *et al.* (1988), S-1,25$(OH)_2$D concentration increased in response to a high-P, low-Ca diet, although after a longer period (4-week) on a similar diet, S-PTH levels increased. Nevertheless, no changes occurred in S-1,25$(OH)_2$D concentration (Calvo *et al.* 1990), which usually increases in response to low Ca intake. An adaptive response to low Ca intake became weaker in a 4-week study. The authors suggested that the usual homeostatic mechanism used when dietary Ca is limited is disturbed by chronic high P intake. A high-P, low-Ca diet increased S-1,25$(OH)_2$D concentration more in men than in women, despite higher S-PTH levels in women (Calvo *et al.* 1988). Unfortunately, S-25(OH)D concentrations were not measured in these studies, as vitamin D status of participants might have affected these results. The finding of Study I suggests that a normal increase in Ca absorption induced by an increase in 1,25$(OH)_2$D as a consequence of a low Ca intake is inhibited by a high P intake. This implies that the normal relationship between Ca intake and Ca absorption is

disturbed in a high-P and low-Ca diet. Presumably, high P intake could decrease active Ca absorption in the long run (Portale *et al.* 1986).

S-Pi is mainly controlled by changes in urinary phosphate (U-Pi) excretion (for review, see Murer *et al.* 2000). In Study I, S-Pi concentration increased in a dose-dependent manner in response to P intake and resulted in a dose-dependent increase in U-Pi excretion. Both PTH and P intake itself can downregulate NPTs in the kidneys (Takeda *et al.* 1999), thereby increasing U-Pi and decreasing urinary Ca (U-Ca) excretions. In Study I, U-Ca excretion decreased at all P doses, but the decrease was significant only at the two highest doses (750 and 1500 mg). The excretions with these two P doses were similar, suggesting that excretion might not diminish after a certain high dietary P intake, but may plateau despite higher S-Pi and S-PTH concentrations, thus resulting in an unfavourable Ca balance. In several earlier studies, high P intake decreased U-Ca excretion in acute and long-term situations (Table 20). The effects on U-Ca occurred after phosphate salts (Calvo and Heath III 1988) or foods with high P content (Calvo *et al.* 1990) were ingested.

6.1.1.2 Bone formation and resorption

A significant decline occurred in serum bone alkaline phosphatase (S-BALP) activity with the 1500-mg P dose, indicating inhibition of bone formation due to high P intake in acute controlled situations (Study I). This finding is in accordance with the results of two previous studies (Kärkkäinen and Lamberg-Allardt 1996, Grimm *et al.* 2001). Kärkkäinen and Lamberg-Allardt (1996) reported S-BALP activity to decrease after a single 1500-mg oral P dose as well as after three separate 500-mg P doses. However, the results of the effects of P intake on bone formation markers published in earlier studies have been conflicting. Bone formation markers have either decreased (BALP, serum procollagen type I carboxyterminal peptide, osteocalcin; Kärkkäinen and 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, Whybro *et al.* 1998). The differences in protocols, e.g. in Ca and P intakes, length of study and differences in the sensitivity of bone metabolism markers, probably explain the discrepant results. However, *in vitro* results of the effects of P on osteoblasts have been shown to stimulate bone matrix formation (Asher *et al.* 1974) and bone mineralization (Bingham and Raisz 1974). These are in contrast to findings in human subjects, as in physiological situations, P stimulates PTH secretion, which, in turn, has been demonstrated to decrease BALP activity within 12 h (Hodsman *et al.* 1993).

High P intake (1500-mg P dose) also increased excretion of the marker of bone resorption (24-h urinary excretion of N-terminal telopeptide of collagen type I corrected for creatinine excretion, U-NTx/U-Cr), indicating increased bone resorption during high P intake (1995 mg/d) (Study I). High dietary P intake increases S-PTH, and PTH is well known to increase bone resorption. In a previous study with a similarly aged group of women with similar Ca intake, no change was found in the serum type I collagen c-

terminal telopeptide (S-CTx) or in the free form of urinary deoxypyridinoline/U-Cr (U-DPD/U-Cr) excretion after a 1500-mg P dose (Kärkkäinen and Lamberg-Allardt 1996). The discrepancy in this earlier study could have been due to neither S-CTx (Garnero *et al.* 1994) nor U-DPD/U-Cr (Rubinacci *et al.* 1999) being very sensitive markers of bone resorption. In different settings, other markers of bone resorption, such as urinary hydroxyproline (U-Hyp), either increased (Calvo *et al.* 1988) or showed no change (Silverberg *et al.* 1986). In studies with no proper control session, acute oral P intakes did not affect the markers of bone resorption (Brixen *et al.* 1992, Renier *et al.* 1992).

6.1.2 Dietary phosphorus sources in foods

The findings of our cross-sectional study (III) suggest that in habitual diets phosphate additives might affect Ca and bone metabolism more negatively than other P sources, as indicated by higher mean S-PTH concentrations among participants consuming phosphate additive-containing foods. Higher consumption of natural P sources, by contrast, was associated with lower S-PTH concentrations. These findings are discussed in greater detail below.

6.1.2.1 Foods containing phosphate additives

Processed cheeses contain both forms of P (natural P and phosphate additives). The lower the fat content in processed cheese, the higher the P content (National Institute for Health and Welfare 2009) originating from phosphate additives (Suurseppä *et al.* 2001). In the present cross-sectional study (III), consumers of processed cheese had higher S-PTH concentrations than non-consumers. This finding might reflect the negative influence of phosphate salts on Ca and bone metabolism found also in earlier intervention (Calvo *et al*. 1988, Calvo *et al.* 1990) and controlled 24-h (Karp *et al.* 2007) studies. In the above intervention studies, diets assembled from common foods, including processed foods with phosphate additives, increased S-PTH concentration in young adults. Phosphate additive-containing foods included processed cheese, instant pudding and cola beverages. In the above-mentioned acute controlled study with healthy women, phosphate additives increased S-PTH concentration in contrast to natural P derived from meat, cheese or whole-grain products. Based on S-Pi concentration and U-Pi excretion, P from phosphate salts and meat appeared to absorb better than P from grain products (Karp *et al.* 2007). Recently, in a short-term study, our results suggested that the consumption of phosphate additive-containing processed cheese increased S-PTH and decreased S-iCa concentrations compared with the natural P-containing fermented cheese (Karp *et al.* 2009b, unpublished data). The study was conducted as a pilot study with only a small number of participants, and thus, additional studies are needed to confirm these findings. In fact, P from phosphate additives may be almost completely absorbed, and the intake of P from such a source represents a larger burden on the human body (Uribarri and Calvo 2003). In Study III, the intake of P from processed cheese was not high relative to total

dietary P intake. Therefore, these findings suggest that P intake, which causes an increase in S-PTH, need not necessarily be high if P is derived from phosphate additives. This might be important information, especially for patients with renal disease. Recently, Sullivan *et al.* (2009) reported that educating kidney patients to avoid foods with phosphate additives resulted in slight improvements in hyperphosphataemia compared with control patients receiving only standard care.

6.1.2.2 Foods containing natural phosphorus

Milk and cheese (excluding processed cheese) are free of phosphate additives, but high in natural P. While P from phosphate additives was associated with higher S-PTH, the effects of natural P on S-PTH were contradictory; those who consumed more milk and cheese had lower mean S-PTH concentrations than those who consumed less (Study III). 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, and, as was found in the controlled study (II), Ca supplementation suppressed higher S-PTH concentrations induced by high P intake. Earlier findings support the present results since in postmenopausal women S-PTH decreased with increasing habitual dietary Ca intake despite simultaneously increasing habitual dietary P intake (Kärkkäinen *et al.* 1998). In addition, consumption of natural P-containing fermented cheese decreased S-PTH concentrations and even decreased bone resorption in an acute study (Karp *et al.* 2007). In milk and cheese, the calcium-to-phosphorus ratio (Ca:P ratio) is ideal, and the importance of a sufficient dietary Ca:P ratio for bone health is supported by the results of earlier epidemiological (Metz *et al.* 1993, Teegarden *et al.* 1998, Basabe *et al.* 2004) and intervention (Calvo *et al*. 1990) studies in humans. However, other factors, such as milk protein intake (Budek *et al.* 2007) or healthy eating habits may potentially be linked to higher milk and cheese consumption, which might cause the favourable effect of natural P sources observed this study (III). Although total Ca intake was used as a covariate, the differences in dietary Ca sources might have further explained the results. Unfortunately, we were unable to collect more exact information on the dietary Ca sources of participants in this cross-sectional study. Moreover, dairy products are also rich in several other nutrients, and optimal skeletal health requires adequate intake of many nutrients, not only Ca (see review by Heaney 2009). In Study III, we investigated milk, cheese and processed cheese, which represent a certain source type of natural P and phosphate additives, while the effects of other foods, e.g. meat or baking products, might be different because dairy products are the only foodstuffs containing large amounts of both P and Ca.

6.1.3 Conclusions about the effects of dietary phosphorus intakes and sources

In the controlled study (I), we found a dose-dependent increase in both S-Pi and S-PTH concentrations in healthy humans due to an increase in dietary P intake. The high P intake (1995 mg/d), corresponding to the mean P intake of Finnish men, negatively affected not only Ca metabolism but also bone metabolism by increasing bone resorption and decreasing bone formation. It is important to note that P was ingested throughout the day, simulating the situation in which food with high P content is consumed. The findings of Study I imply that foods with high P content may cause transient hyperparathyroidism in healthy individuals, at least when Ca intake is low. These results further indicate that the normal relationship between Ca intake and Ca absorption is disturbed in a high-P, low-Ca diet. Moreover, in the cross-sectional study, higher total habitual dietary P intakes were associated with higher S-PTH and lower S-iCa concentrations in healthy Finnish women. The results of these two different study designs suggest that high dietary P intake has more detrimental effects than low P intake on Ca and bone metabolism. Furthermore, not only P doses but also P sources have an impact, as in the habitual diets of healthy individuals, foods containing phosphate additives had stronger negative associations with Ca metabolism than foods containing natural P. These associations were seen as higher S-PTH concentrations among those who consumed phosphate additive-containing foods. This difference may be due to the different bioavailability of P from phosphate salts and natural P sources. Because of the high dietary P intake and the current upward trend in consumption of processed foods in Western countries, findings from both studies may have important public health implications. The intakes of phosphate additives and total P have been shown to rise due to increasing consumption of fast foods and snacks. 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.

6.2 Combined effects of dietary calcium and phosphorus intakes on calcium and bone metabolism

In the controlled 24-h study (II), when P intake (1850 mg/d) was 3-fold above the current recommendations, by increasing the daily Ca intake from 480 mg to 1080 mg and further to 1680 mg, several beneficial effects on Ca and bone metabolism were noted. However, not even a high Ca intake (1680 mg/d) could affect bone formation when P intake was excessive. In the cross-sectional study (IV), low habitual dietary Ca:P ratios (Ca:P molar ratio $\leq$0.50) had unfavourable associations with Ca and bone metabolism in healthy women with an adequate Ca intake. In fact, the lowest quartile, with a Ca:P molar ratio $\leq$0.50, differed significantly from the other quartiles by being associated with both increased S-PTH concentration and increased U-Ca excretion. None of the women in Study IV achieved the suggested dietary Ca:P molar ratio of 1, although their habitual dietary Ca intakes were in general adequate. This is discussed in greater detail below.

6.2.1 Serum PTH concentration and other calcium metabolism markers

While dietary P intake increases S-PTH concentration by decreasing S-iCa concentration (Herfarth *et al.* 1992b) and by directly affecting PTH secretion (Slatopolsky *et al.* 1996), Ca administration has been demonstrated to decrease S-PTH in young adults (Kärkkäinen *et al*. 2001, Sadideen and Swaminathan 2004) via an increase in S-iCa (Herfarth *et al.* 1992b). In the controlled study (II), S-PTH concentration decreased in a dose-dependent manner with increasing Ca intake (1060 and 1680 mg), indicating that Ca supplementation can reduce the rise in PTH induced by higher dietary P intake (1850 mg/d). 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 and the low Ca intake on S-PTH. In habitual diets (Study IV), higher S-PTH concentrations were found in participants with low dietary Ca:P ratios (Ca:P molar ratio ≤0.50). While the mean intake of P in all quartiles of this cross-sectional study (IV) was over 2-fold higher (>1200 mg/d) than dietary guidelines (National Nutrition Council 2005), the mean intake of Ca was slightly below recommended levels (National Nutrition Council 2005) in the lowest quartile only (742 mg/d). Excluding the lowest quartile revealed similar associations with S-PTH in the other quartiles. The finding of higher mean S-PTH only in the lowest quartile (Study IV) supports the importance of higher dietary Ca:P ratios and the vital role of adequate Ca intake simultaneously with high dietary P intake in habitual diets.

S-iCa concentration increased in a dose-dependent manner with increasing Ca doses in the controlled study (II). In previous intervention studies, a high intake of P decreased S-iCa concentration in females (Calvo *et al.* 1988, Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996) and males (Portale *et al.* 1987). However, Grimm *et al.* (2001) reported that S-iCa concentration did not change even with 3000 mg of P, probably due to the simultaneously high Ca content (1995 mg) of the diet. Guillemant and Guillemant (1993) showed a dose-response relationship between 500- and 1500-mg Ca doses and S-iCa concentration. However, with higher doses of Ca (1000 and 2000 mg) S-iCa increased in a similar manner (Herfart *et al.* 1992a). In Study II, we demonstrated the dual effects of high P and varying Ca intakes in human subjects and found that the lower the Ca:P ratio and Ca intake, the lower the S-iCa concentration and the higher the S-PTH concentration.

No significant differences emerged in S-Pi concentrations in the controlled study (II), which might be due to the constant P intake 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 during both 600-mg and 1200-mg Ca dose sessions. 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 (Whybro *et al.* 1998, Grimm *et al.* 2001). This is probably due to diminished P absorption because of the formation of the Ca-phosphate complex in the gut. However, contradictory reports exist of

the effects of P on Ca absorption (Zemel and Linkswiler 1981, Spencer *et al.* 1986, Heaney and Recker 1994), and relatively few studies are available. Ca, on the other hand, has been shown to decrease P absorption (Heaney and Nordin 2002), and in renal disease, Ca compounds are used to bind dietary P in the gut (Nolan and Qunibi 2003).

U-Pi excretion decreased in a dose-dependent manner with increasing Ca intake in the controlled study (II). Oral Ca intake ($CaCO_3$) has been demonstrated to diminish U-Pi excretion (Mortensen and Charles 1996). This is due to increased S-iCa concentration, which in turns decreases S-PTH concentration and leads to lower U-Pi excretion, even without any alterations in P intake. The influence of Ca intake was also seen in U-Ca excretion, which increased dose-dependently with Ca doses (Study II). Because P intake was maintained at exactly the same level for all study days, the dose–response effect of Ca on U-Pi and U-Ca excretion could be determined. However, an unexpected physiological increase in 24-h U-Ca excretion was observed in the lowest Ca:P ratio quartile in the cross-sectional study (IV). This was unexpected since the mean dietary Ca intake and Ca:P ratios in this quartile were the lowest and the mean S-PTH concentration was the highest. U-Ca excretion was anticipated to be the highest in the 3rd and 4th quartiles, as habitual dietary P intake was high in all quartiles, but habitual dietary Ca intake was significantly higher in the 3rd and 4th quartiles only. After excluding the lowest quartile, the other quartiles were highly similar in U-Ca excretion findings. U-Ca excretion is an important determinant of Ca retention in the healthy human body. In normal physiological conditions, elevated S-PTH leads to decreased U-Ca excretion, while in primary hyperparathyroidism, an increase in U-Ca excretion is seen (Silverberg and Bilezikian 2008). Low Ca intake and high U-Ca excretion may reduce Ca accretion in bone of young adults during growth, having a negative impact on skeletal development (Matkovic *et al.* 1995). The findings of Study IV suggest that low dietary Ca:P ratios in habitual diets somehow interfere with the homeostasis of Ca metabolism. Elevated U-Ca excretion might reflect increased bone resorption, as an increase in S-PTH due to a low Ca:P ratio would be expected to increase bone resorption, and some of the extra Ca, which has been released from bone, would be excreted in urine. Thus, in habitual diets with excessive dietary P intake, an adequate dietary Ca intake might be needed to overcome the interfering effects of low Ca:P ratios on Ca excretion. Nevertheless, increasing dietary Ca intake far beyond nutritional recommendations is not advisable because this might result in other health risks (Whiting and Wood 1997). A very high Ca intake has been associated with increased risk of kidney stones, negative Ca-mineral interactions and vascular mineralization (Whiting and Wood 1997, Winzenberg and Jones 2008).

6.2.2 Bone formation and resorption

No significant changes occurred in bone formation, as indicated by S-BALP activity in Study II. In earlier reports, the effects of high P intake on bone formation markers have been contradictory, with bone formation markers showing a decrease (S-BALP, serum procollagen type I carboxyterminal peptide, osteocalcin) (Kärkkäinen and Lamberg-

Allardt 1996, Grimm *et al.* 2001), an increase (osteocalcin) (Brixen *et al.* 1992) or no change (osteocalcin) (Calvo *et al.* 1990). However, in these studies dietary Ca intake varied, being low (Calvo *et al.* 1990, Kärkkäinen and Lamberg-Allardt 1996), high (Grimm *et al.* 2001) or unknown (Brixen *et al*. 1992). The acute effect of Ca on bone formation has also yielded inconsistent findings (e.g. Kärkkäinen *et al.* 2001). In an uncontrolled acute study, Ca infusion did not affect osteocalcin (OC) within 50 min (Bergenfelz and Ahren 1992), although extracellular Ca concentration has been demonstrated to influence osteoblasts *per se* (Chattopadhyay *et al.* 1996). When examining the effect of P on bone formation in Study I, S-BALP activity decreased with increasing P doses. In Study II, P intake was maintained at the same level (1850 mg/d) on each experiment day, and the effect of 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 the finding of an unchanged bone formation rate is that high Ca intake cannot counteract the effects of higher-than-recommended P intakes. If this interpretation is correct, the effects of increasing intake of dietary P on bone may be as alarming as previously summarized by Sax (2001).

Although there was no change in bone formation, Ca supplementation decreased bone resorption, as indicated by the decreased excretion of U-NTx/U-Cr. Already in Study I, bone resorption (U-NTx/U-Cr) increased with a high-P (1995 mg/d) and low-Ca (250 mg/d) treatment. The highest P intake of Study I corresponds to the P intake in Study II. Therefore, the decrease in bone resorption in Study II indicates that Ca supplementation can diminish the effects of a P intake that is 3-fold higher than the recommended 600 mg/d (National Nutrition Council 2005). In other Ca administration studies, increased Ca intake was found to decrease bone resorption in adolescent girls (Wastney *et al.* 2000), young adults (Kärkkäinen *et al.* 2001, Sadideen and Swaminathan 2004) and male athletes (Guillemant *et al.* 2004), while the effects of high P intake on bone metabolism have been demonstrated to be the opposite in a controlled P study (Kärkkäinen and Lamberg-Allardt 1996) and an 8-day intervention study (Calvo *et al.* 1988).

6.2.3 Determinants of habitual dietary calcium-to-phosphorus ratios

Speculation has arisen whether the dietary Ca:P ratio is clinically significant in adult humans (Food and Nutrition Board 1997, Sax 2001) since in adult diets the Ca:P ratio varies, being the highest in dairy products. While P is present in a wide range of foods, around 75% of Ca in Finland comes from dairy products (Paturi *et al.* 2008). Although some foods rich in P are also good sources of Ca, e.g. dairy products, many others contain very little Ca. Furthermore, P is added to foods as a phosphate additive, further increasing already high P intake. If the habitual diet lacks dairy products, the dietary Ca:P ratio will easily be low. Very low Ca:P weight ratios (≤0.25) have been reported in diets of young girls and boys in Poland (Chwojnowska *et al.* 2002) as well as in teenagers and young adults in USA (Calvo 1993). However, whether it is necessary to reach a Ca:P molar ratio of 1 (e.g. SCF 1993, National Nutrition Council 2005) in diets is unknown. In the habitual

diets of Study IV, even when the dietary Ca intake among participants was mostly adequate or high (mean Ca intake 1056 mg/d), none of the participants achieved the suggested Ca:P molar ratio of 1. This was mainly because of the excessive P content in their habitual diets, rather than a low dietary Ca intake, as mean dietary P intake exceeded 2.4-fold and mean dietary Ca intake 1.3-fold the Finnish nutritional recommendations for P (600 mg/d) and Ca (800 mg/d) (National Nutrition Council 2005). Results of Study IV also imply that a cut-off Ca:P ratio may exist, below which the effects on mineral metabolism and bone health are more severe. In this study, such a cut-off Ca:P molar ratio was 0.51. However, higher Ca:P ratios might be needed if dietary Ca intake drops markedly below nutritional recommendations.

6.2.4 Conclusions about the combined effects of dietary calcium and phosphorus

We demonstrated in the experimental human study (II) that when dietary P intake is 3-fold above the dietary guidelines, oral Ca intake decreases S-PTH concentration and bone resorption, both of which have been induced by increased P intake. However, even high Ca intake (in total 1680 mg/d) could not change the bone formation activity, which was demonstrated to decrease due to high P intake in healthy females in the controlled study (I). It is noteworthy that the P intake (1850 mg/d) in Study II corresponds to the average estimated dietary intake in Western countries, and all ingested P originated from normal foods. The higher intakes of Ca (1080 and 1680 mg/d), in turn, are beyond the reach of Western diets in many countries. These results suggest that higher doses of Ca than those used in our study are needed to prevent the negative effect of high P intake, although lower doses also offer several favourable effects. The importance of dietary Ca:P ratios was also found in habitual diets (Study IV), as low dietary Ca:P molar ratios ($\leq$0.50) were associated with both increased S-PTH and increased U-Ca excretion in healthy women with an adequate dietary Ca intake. These findings suggest that such a low Ca:P ratio may negatively affect bone health, as higher S-PTH concentrations in healthy humans ensure that Ca remains inside the body and is not excreted in urine. Results of these two different kinds of study designs indicate that concerns regarding high P consumption are warranted. Because low habitual dietary Ca:P ratios are common in Western diets, more attention should be focused on decreasing excessively high dietary P intake and increasing Ca intake to recommended levels. In Western diets, dairy product consumption will easily ensure adequate Ca intake. High dietary P intake, in turn, will be reduced by restricting the consumption of highly processed foods and increasing the consumption of raw and unprocessed foods.

6.3 Strengths and limitations of the studies

6.3.1 Study design

Firstly, we demonstrated in a controlled study design (I) that high P intake has adverse effects on Ca and bone metabolism. Secondly, we investigated in another controlled study (II) whether the negative effects of high P intake, equal to the highest P intake in Study I, could be reduced by increasing Ca intake. Thirdly, the results derived from these two controlled studies prompted us to continue and expand the investigations to habitual diets (Studies III and IV). Thus, some findings from controlled studies were also investigated in cross-sectional study settings to determine whether the effects found in controlled diets could also be seen as similar associations in habitual diets.

6.3.1.1 Sample size

In each study, the main outcome variable was S-PTH. Power calculation based on S-PTH concentration (expected difference between quartiles in mean S-PTH concentration 12 ng/l), assuming 90% power with α=0.05, indicated that a sample size of 11 in each group was adequate (Kärkkäinen *et al.* 1998). The number of recruited participants in the controlled studies (I and II) and the size of quartiles and groups in the cross-sectional study designs (III and IV) were based on this calculation. Although we found that P and Ca significantly affected bone resorption and formation markers in both of the controlled studies, a larger variation existed in the bone markers than in the S-PTH concentrations between and within individuals. Therefore, in Studies I and II, larger sample sizes might have detected all of the changes in these markers. In forthcoming studies where bone markers are measured, a power calculation based on these markers is advisable. This will ensure that a study will not lack the intended power, and sample size will be optimal, as both too small and too large sample sizes are considered to be unethical.

6.3.1.2 Study design in controlled studies

In the controlled studies (I and II), to assure that subjects consumed all of the meals they were offered on each study day, the subjects ate their meals, with the exception of supper, in the research unit. These studies were performed with Ca and P doses normal and achievable in Western diets, and Ca and P were ingested throughout the day, simulating the situation in which food with high P or Ca content is consumed. To ensure that vitamin D status was as similar as possible between subjects, both studies were conducted before the summer months. We did not measure vitamin D status (S-25(OH)D) of our subjects, as each subject served as her own control, and the order of the study sessions was randomized. In addition, a low S-25(OH)D status could have affected PTH results of subjects (Lamberg-Allardt *et al.* 2001), but none of subjects had abnormally high fasting

S-PTH concentrations, which would have indicated severe vitamin D deficiency. In addition, in keeping Ca (Study I) and P (Study II) intakes constant and escalating the doses of P (Study I) and Ca (Study II), we were able to investigate the dose-response effects of P in Study I and Ca in Study II.

6.3.1.3 Study design in cross-sectional studies

Unlike in experimental studies, in cross-sectional studies, subjects and phenomena are more intact and discrete. However, cross-sectional studies also have some advantages, e.g. lower drop-out rates and subjects whose behaviour is less affected by study designs. Cross-sectional study designs need careful planning, but the cross-sectional studies here (III, IV), although well-planned nutritional studies, were not originally designed as P studies. The original study was carefully designed for vitamin D investigation (Lamberg-Allardt *et al.* 2001). While the information collected (4-day food records, blood and urine samples, background information) in this original study, upon which substudies III and IV were based, was adequate, had this cross-sectional study been conducted nowadays, we would have been able to gather more specific information on P intakes and address the research questions in more detail. This is due to the newly developed and validated food frequency questionnaire (FFQ), determining the total P intakes and separating the intakes of P from different P sources (Kemi *et al.* 2009b, unpublished data). The FFQ contains a total of 100 P-containing food items, selected for the FFQ based on their contribution to the population intake of P. The FFQ has been validated with 4-day food records. Pearson correlation coefficient between FFQ and the 4-day food records was 0.70 ($p<0.0001$). The total mean P intake was slightly higher (~100 mg/d) based on the FFQ than on the 4-day food records. Furthermore, due to the outdated computer-based program (UNIDAP), we were unable to collect more exact information on the dietary P and Ca sources of participants. Therefore, all 4-day food records were recoded and analysed, and the participants´ milk, cheese and processed cheese consumptions and the intake of dietary P from these foods were manually calculated by using the Fineli 2006 database (National Institute for Health and Welfare 2009).

The data of the original cross-sectional study are 10 years old and therefore might not completely reflect the P rich diets consumed nowadays. However, the average Ca and P intakes of our participants corresponded well with the average Ca and P intakes in Finnish females in 2007 (Paturi *et al.* 2008). Moreover, in Study III, foods investigated included milk, cheese and processed cheese, and the P content of these foods is thought to be constant over the last 10 years. In production of processed cheese, phosphate additives were similarly used 10 years ago and today. However, during the last decades the consumption of processed foods and low-fat products, which in some cases contain more phosphate additives than products with normal fat content (Suurseppä *et al.* 2001), has increased, which has resulted in higher intake of total P and P from phosphate additives. Worldwide, it is a challenging task to update the food composition tables and to determine the actual P content of foods, especially those containing phosphate additives.

6.3.1.4 Study participants

In the cross-sectional studies (III, IV), participants represented a subgroup of randomly selected 31- to 43-year-old Finnish women. These women had participated in an earlier National FINRISK survey (FINDIET 1997 Study Group 1998), and thus, might had become more familiar with being study subjects, which may have affected how they kept a 4-day food record. Volunteers are usually more health-orientated, healthier and better educated than those who not participate in studies. This influences the generalizability of results. However, background information revealed that Ca, P and energy intake of participants corresponded well with the average intakes of Finnish females found in two separate FINDIET surveys (Männistö *et al.* 2002, Paturi *et al.* 2008).

Based on earlier findings (Calvo *et al.* 1990), differences exist in metabolic responses between men and women. Thus, to evaluate both sexes in the same study is not rational. Moreover, young and premenopausal women were investigated, as P supplementation has been reported to increase S-PTH concentration more in women aged over 50 years than in younger women (Renier *et al.* 1992). While men have a higher total P intake and presumably a higher P intake from phosphate additives due to greater consumption of foods containing P additives, women consume more low-fat products. These products, in some cases, contain more phosphate additives than products with normal fat content. In this thesis, women served as study subjects since before menopause women are more vulnerable than men to developing osteoporosis due to a lower peak bone mass (Melton *et al.* 2003, Seeman *et al.* 2003). Another important practical factor is that women are usually more eager than men to participate in studies.

6.3.2 Methods

6.3.2.1 Dietary assessment

As almost all foods contain P, the calculation of dietary P intake by 4-day food record is more reliable than calculation of, for instance, dietary vitamin D intake by the same method. However, relative to the 4-day food record, the food frequency questionnaire (FFQ) would have been easier method for both subjects and researchers to use. The FFQ also divides the sources of P and includes sources that are not consumed every day (Kemi *et al.* 2009b, unpublished data), as the questionnaire is retrospective, exploring the previous month. Furthermore, subjects usually remember volumes better than weights (Willet 1998). In fact, the need for FFQ development arose during the course of these studies. Earlier, the FFQ in determining the intake of dietary vitamin D and Ca was validated with a dietary recall method by our research group (Lamberg-Allardt *et al.* 2001). In Study III, the FFQ would have divided consumers and non-consumers of processed cheese more precisely than a 4-day food diary, as 4 days is a short period in which to recognize consumers and non-consumers of a certain food item.

In each study, the dietary contents of the 4-day food records were calculated with the computer-based programs based on the same food composition database (Fineli®) (National Institute for Health and Welfare 2009). It is noteworthy that mostly the P contents of foods presented in Fineli were analysed before 1990. Some P contents have been calculated from recipes using data on raw materials; thus, the mineral content of these foods is not analysed from authentic, processed foodstuffs. All in all, an urgent need exists for updating P contents of foods by laboratory analyses. Our research group in cooperation with the Division of Food Chemistry, University of Helsinki, have recently launched this type of studies, and some of our results have been presented in recent congresses in 2009 (Itkonen *et al.* 2009, Karp *et al.* 2009a).

6.3.2.2 Laboratory methods

Due to restricted financial support, it was possible to measure only one bone formation marker and one bone resorption marker in Studies I and II. Measurements of bone markers in Studies III and IV were impossible, as the blood and urine samples no longer existed in 2006. To obtain more reliable information on bone resorption and formation, at least two markers of each would have been ideal to monitor. However, as Study II was a sequel to Study I, we diminished a source of error by measuring the same bone formation and resorption markers in these studies, improving the comparability of these two studies. BALP was chosen as a bone formation marker because Kärkkäinen and Lamberg-Allardt (1996) demonstrated a rapid decrease in BALP activity induced by P load. At the time that these studies were conducted, few reliable analyses were available for fibroblastic growth factor 23 (FGF-23) measurements and their costs exceeded our study budgets. We therefore could not measure serum FGF-23 concentrations, and gained no detailed information about the effects of P doses and sources on FGF-23.

7 Conclusions and future prospects

This thesis contributes novel information related to the effects of dietary phosphorus (P) and the combined effects of P and calcium (Ca) on Ca and bone metabolism in healthy individuals. It is already well established that high P intake is detrimental for patients with impaired renal functioning, but the effects of high P intake in healthy humans have been investigated seldom. In this thesis, an excessively high P intake, which is common in Western countries, was observed to negatively affect Ca and bone metabolism. Moreover, P doses affected Ca and bone metabolism in a dose-dependent manner, and P sources also differed in their effects on the essential regulator of Ca and bone metabolism. Finally, we demonstrated that by increasing dietary Ca intake the negative effects of a high P intake could be reduced. However, based on the findings of the controlled study, even a high dietary Ca intake could not completely overcome all of the negative effects caused by a high dietary P intake.

Specific findings of each study were as follows:

Study I

In a controlled study with healthy young women, the oral intake of P in doses comparable with normal dietary intakes (495, 745, 1245 and 1995 mg/d) with a low Ca intake (250 mg/d) increased serum parathyroid hormone (S-PTH) concentration in a dose-dependent manner. The highest P dose had the most negative effects, as with the highest dose there was also a decrease in serum ionized Ca (S-iCa) concentration and bone formation and an increase in bone resorption. Furthermore, a high P intake (1995 mg/d) inhibited the increase in serum $1,25(OH)_2D$ in response to a low dietary Ca intake, implying that the normal relationship between Ca intake and Ca absorption is disturbed in diets high in P and low in Ca. This study showed that P has a dose-dependent effect on S-PTH and increases PTH secretion significantly when Ca intake is low. An acutely high P intake adversely affects bone metabolism by decreasing bone formation and increasing bone resorption.

Study II

In a controlled study of healthy 20- to 40-year-old women with a dietary P intake that was 3-fold above the dietary guidelines (1850 mg/d), by increasing the Ca intake from 480 mg/d to 1080 mg/d and then to 1680 mg/d, the S-PTH concentration decreased, the S-iCa concentration increased and bone resorption decreased dose-dependently. This study showed that a dietary Ca intake above the recommended level offers several advantages in preventing the negative effects of a high P intake. However, not even the highest Ca intake (1680 mg/d) could counteract the effect of high dietary P on bone formation, as indicated by unchanged bone formation activity.

Study III

In a cross-sectional study with healthy 31- to 43-year-old women, a high habitual dietary P intake was associated with increased S-PTH and decreased S-iCa

concentrations. These results are in line with observations in our controlled short-term study. Furthermore, in the habitual diets, phosphate additives, unlike other P sources, were associated with higher mean S-PTH concentrations. The association of S-PTH with natural P was the opposite of that with foods containing phosphate additives. Thus, these results suggest that P sources might differ in their effects on the central regulator of Ca and bone metabolism, parathyroid hormone.

Study IV

In a cross-sectional study of healthy 31- to 43-year-old women with an adequate Ca intake, low habitual dietary Ca:P ratios (Ca:P molar ratio ≤0.50) were associated with both higher S-PTH and U-Ca levels. Interestingly, the lowest Ca:P quartile with a Ca:P molar ratio ≤0.50 differed from all other quartiles by having the most deleterious associations with Ca metabolism. These results imply that a cut-off Ca:P ratio may exist that is lower than the suggested Ca:P molar ratio of 1, below which the effects on mineral metabolism and bone health are more severe. None of the study subjects achieved the suggested dietary Ca:P molar ratio of 1.

A question arising from the findings of this thesis is how to reduce the current excessively high dietary P intake. The answer to this is not straightforward, as the current P intakes are not well known since the use of phosphate additives has not been taken into consideration when establishing food composition databases. In the future, food composition databases should be updated and the actual P contents of foodstuffs determined by laboratory analyses. In addition, it is impossible for an individual to know how much P foods contain because P is not included in the compulsory food ingredient list. While waiting for these issues to be addressed, one can reduce dietary P intake by restricting the consumption of highly processed foods and increasing the consumption of raw or unprocessed foods.

As there are still many open questions concerning the effects of high P intake on bone health, we will continue to investigate whether dietary P intakes and different P sources affect bone mass and structure in adult Finns. However, nowadays, when considering public health, it is not only osteoporosis but also other public health diseases in which a high P intake may play an important role in a negative sense. An alarming rise has been seen among Western populations in the incidence of type 2 diabetes, a major cause of end-stage renal disease, in which dietary P restriction is a part of the treatment. Among patients with end-stage renal disease and diabetes, vascular calcification correlates highly with cardiovascular disease mortality. Recent results suggest that an excessive P intake may be involved in this vascular calcification process, even in healthy humans. Therefore, in the near future, our studies will expand to investigate the relationship between dietary P and vascular calcification in Finnish adults.

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