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Dietary phosphorus sources and their acute effects on mineral metabolism in healthy women

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ACADEMIC DISSERTATION

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To my family

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Suomalaisten fosforin saanti on runsasta verrattuna ravitsemussuosituksiin: naisilla saanti on keskimäärin 1350 mg/vrk ja miehillä 1800 mg/vrk suosituksen ollessa aikuisväestölle 600 mg/vrk. Runsas fosforin saanti on haitallista kroonisesta munuaisten vajaatoiminnasta kärsiville, mutta viimeaikaisten tutkimusten perusteella se saattaa lisätä myös sydän- ja verisuonitautien sekä osteoporoosin riskiä väestötasolla. Fosforia saadaan luontaisesti useista eri lähteistä kuten maidosta, lihasta, viljasta, palkoviljasta ja kananmunista. Fosforin saanti on kuitenkin lisääntynyt etenkin elintarviketeollisuuden kasvaneen lisäainefosfaattien käytön seurauksena. Tieto elintarvikkeiden fosforipitoisuuksista on puutteellista; esimerkiksi elintarvikkeiden koostumustietokannat eivät ota huomioon lisäaineiden sisältämää fosforia. Elintarvikkeiden lisäaineissa fosfori on epäorgaanisessa muodossa, josta sen epäillään imeytyvän käytännössä kokonaan. Sen sijaan useissa kasvikunnan tuotteissa fosfori esiintyy osana fytaattia, josta sen imeytyvyys näyttäisi olevan selvästi heikompaa kuin eläinkunnan tuotteista. Kuitenkin elintarvikkeen prosessointi esim. hapattamalla tai kuumentamalla voi hajottaa osan fytaatista, jolloin fosforiakin imeytyy enemmän kuin käsittelemättömästä tuotteesta. Tutkimustietoa fosforin imeytyvyydestä ihmisillä on erittäin vähän. Tässä väitöskirjassa tutkittiin elintarvikkeiden fosforipitoisuuksia sekä verrattiin eri fosforilähteiden vaikutuksia mineraaliaineenvaihduntaan terveillä nuorilla naisilla.

Ensimmäisessä ja toisessa osatyössä määritettiin fosforin saannin kannalta keskeisten, omassa tuoteryhmässään yleisimmin käytettyjen tuotteiden kokonais- ja liukoisen fosforin pitoisuudet sekä vertailtiin liukoisen fosforin osuutta kokonaisfosforista eri elintarvikkeiden välillä. Liukoisen fosforin määrityksessä näytteitä käsitellään ruoansulatusta matkien ennen analysointia. Ensimmäisessä osatyössä määritettiin yhteensä 21 kasviperäisen ruoan ja juoman fosforipitoisuudet. Niiden joukossa suurin kokonaisfosforipitoisuus oli kuorimattomilla seesaminsiemenillä (667 mg/100 g), joilla liukoisen fosforin pitoisuus oli samanaikaisesti pienin (6%). Kolajuomilla ja oluella liukoisen fosforin osuus kokonaisfosforista oli 87-100% (13-22 mg/100 g). Viljatuotteista suurin kokonais- (216 mg/100 g) ja liukoisen fosforin osuus (100%) oli teollisilla muffineilla, jotka sisältävät natriumfosfaattia kohotusaineena. Palkoviljojen keskimääräinen liukoisen fosforin määrä oli 83 mg/100 g (38% kokonaisfosforista). Tutkimuksen perusteella fosforin imeytyvyys kasvikunnan tuotteiden välillä voi vaihdella paljon. Huolimatta suuresta kokonaisfosforipitoisuudesta, palkoviljatuotteet voivat olla melko huonoja fosforilähteitä. Lisäainefosforia sisältävissä tuotteissa liukoisen fosforin osuus oli huomattavan suuri, mikä tukee aiempaa käsitystä fosforin tehokkaasta imeytyvyydestä epäorgaanisista fosfaattiyhdisteistä.

Toisessa osatyössä määritettiin yhteensä 21 liha- ja meijerituotteen kokonais- ja liukoisen fosforin pitoisuus. Suurimmat kokonais- ja liukoisen fosforin pitoisuudet todettiin sulatejuustoilla sekä kovilla, kypsytetyillä juustoilla (kokonaisfosforipitoisuus 529-892 mg/100g), matalimmat puolestaan maidolla ja raejuustolla (108-146 mg/100g). Sekä

kokonais- että liukoisen fosforin pitoisuudet olivat matalammat makkaroilla ja leikkeleillä kuin sulate- ja kypsytetyillä juustoilla. Broilerin, sian- ja naudanlihan sekä kirjolohen kokonaisfosforipitoisuudet (199-232 mg/100g) olivat suunnilleen samalla tasolla, mutta liukoisen fosforin pitoisuudessa oli enemmän vaihtelua (147-207 mg/100g). Tässäkin tutkimuksessa todettiin lisäainefosforia sisältävillä tuotteilla liukoisen fosforin osuuden olevan suuri. Fosfori-proteiinisuhteen sekä natriumpitoisuuden perusteella raejuusto ja lisäaineettomat lihat soveltuvat munuaispotilaille paremmin kuin sulatejuustot, kovat kypsytetyt juustot, leikkeleet tai makkarat. Eläinkunnan tuotteista fosfori vaikuttaisi myös imeytyvän paremmin kuin esimerkiksi palkoviljasta.

Kolmas ja neljäs osatyö olivat ajan ja ravinnon suhteen kontrolloituja interventiotutkimuksia nuorilla naisilla. Jokainen tutkittava toimi itse itsensä kontrollina, ja tutkimuspäivien järjestys oli satunnaistettu. Kunkin 24 tunnin tutkimusjakson aikana kerättiin vuorokausivirtsanäytteet ja otettiin 4 (osatyö III) tai 6 (osatyö IV) verinäytettä, joista määritettiin mineraaliaineenvaihdunnan merkkiaineita. Kolmannessa osatyössä tutkittiin eri fosforilähteiden akuutteja vaikutuksia mineraaliaineenvaihduntaan 15 terveellä nuorella naisella. Kontrollijaksolla fosforin (500 mg) ja kalsiumin (250 mg) saanti oli niukkaa. Muilla jaksoilla fosforin saanti oli n. 1500 mg, josta 1000 mg saatiin vaihdellen lihasta, viljasta, juustosta tai lisäainefosforiseoksesta. Tutkimusruokavaliot koostettiin lisäainefosforiseosta lukuun ottamatta tavallisista elintarvikkeista. Seerumin lisäkilpirauhashormonipitoisuutta (S-PTH) nosti kontrollipäivään verrattuna ainoastaan lisäainefosforiseos. Luun aineenvaihdunnan merkkiaineiden perusteella liha lisäsi sekä luun hajoamista että sen muodostumista. Juusto laski seerumin PTH-pitoisuutta ja vähensi luun hajoamista. Vilja nosti seerumin fosfaattipitoisuutta vähemmän kuin muut fosforilähteet, mikä viittaa siihen, että viljan fosfori on huonommin imeytyvää kuin muista fosforilähteistä peräisin oleva fosfori. Fosforilähteiden vaikutukset mineraaliaineenvaihduntaan poikkesivat siis toisistaan johtuen fosforin erilaisesta imeytyvyydestä ja fosforilähteiden sisältämistä muista ravintoaineista.

Neljännessä osatyössä vertailtiin kahden eri lisäainefosforiyhdisteen, mono- ja polyfosfaatin, vaikutuksia fosfori- ja kalsiumaineenvaihdunnan merkkiaineisiin 14 terveellä nuorella naisella. Jokaisella tutkimusjaksolla koehenkilöt saivat kolme annosta joko mono- tai polyfosfaattia tai lumevalmistetta aterioiden yhteydessä. Molemmat fosfaattiyhdisteet sisälsivät 1500 mg fosforia/vrk, ja ruokavalio jokaisella jaksolla oli täysin sama. Molemmat fosfaattiyhdisteet nostivat seerumin PTH- ja fosfaattipitoisuutta sekä lisäsivät fosfaatin eritystä virtsaan. Polyfosfaatti vähensi kalsiumin eritystä virtsaan monofosfaattia voimakkaammin, joten se saattaa sitoa kalsiumia suolessa monofosfaattia enemmän. Molemmat fosfaattiyhdisteet vaikuttaisivat imeytyvän yhtä tehokkaasti ja nostavan PTH-konsentraatiota, mikä pitkään jatkuessaan on haitallista luustolle.

Väitöskirjan tulokset tukevat aiempaa käsitystä siitä, että fosforilähteiden välillä on eroja imeytyvyydessä ja vaikutuksessa mineraaliaineenvaihduntaan. Tällä on merkitystä kroonisesta munuaisten vajaatoiminnasta kärsivien ruokavaliossa, ja ehkä myös laajemmalti, mikäli runsaan fosforin saannin merkitys myös sydän- ja verisuonitautien ja osteoporoosin etiologiassa vahvistuu. Lisäainefosfori näyttää imeytyvän tehokkaimmin ja

ainakin lyhytkestoisesti vaikuttavan luun aineenvaihduntaan negatiivisesti. Siksi lisäainefosforipitoiset tuotteet voivat olla haitallisimpia fosforirajoitteisessa ruokavaliossa verrattuna luontaisesti fosforia sisältäviin ruokiin. Fosforilähteen vaikutukset elimistössä riippuvat myös sen sisältämistä muista ravintoaineista; terveillä koehenkilöillä kypsytetyn juuston vaikutus mineraaliaineenvaihduntaan oli positiivinen johtuen juuston sisältämästä kalsiumista. Fosfori-proteiinisuhteen perusteella fosforirajoitteiseen ruokavalioon vaikuttaisivat soveltuvan parhaiten lisäainefosfaatittomat palkovilja- ja leivontatuotteet sekä eläinkunnan tuotteista raejuusto ja lisäainefosfaatittomat lihat.

Abstract

The phosphorus (P) intake in Finland exceeds the recommended levels, being on average 1350 mg in women and 1800 mg in men. A high P intake is deleterious to chronic kidney disease (CKD) patients, but based on more recent data, it has also been associated with an increased risk of osteoporosis and cardiovascular diseases in the general population. In addition to several natural sources of P (especially dairy products, meats, whole grains, legumes, and eggs), the use of P additives in the food industry is common and further increases P intake. P from inorganic additives is believed to be absorbed almost completely. However, the food composition databases do not adequately take into account the current use of P additives. In several plant foods, P exists as a component of phytic acid; less P from plants seems to be absorbed than from animal foods. Processing of a food, e.g. by fermenting or heating, may degrade phytate, improving P absorption. Data on P content of foods are needed to facilitate research on P intake and its health effects. Data on absorbability of P from different food sources and their effects on organs are also lacking. In this thesis, P contents of foods were analysed and the effects of different P sources on mineral metabolism were compared in healthy young female subjects.

In Studies I and II, the objective was to measure both total phosphorus (TP) and *in vitro* digestible phosphorus (DP) contents of selected foods and to compare the amounts of TP and DP and the proportion of DP to TP among different foods. The most popular national brands were chosen for analysis. TP and DP contents of 21 products were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). In DP analysis, samples were digested enzymatically in principle in the same way as in the alimentary canal before P analyses. In Study I, TP and DP contents of 21 foods and beverages of plant origin were analysed. Among these, the highest amount of TP (667 mg/100 g) was found in sesame seeds with hull, which also had the lowest proportion of DP to TP (6%). In cola drinks and beer, by contrast, the proportion of DP to TP was 87-100% (13-22 mg/100 g). In cereal products, the highest TP content (216 mg/100 g) and DP proportion (100%) were present in industrial muffins, which contain sodium phosphate as a leavening agent. Legumes contained an average DP content of 83 mg/100 g (38% of TP). These results suggest that the absorbability of P may differ substantially among different plant foods. Despite high TP content, legumes may be a relatively poor P source. In foods containing phosphate additives, the proportion of DP is high, which supports previous conclusions of the effective absorbability of P from P additives.

In Study II, TP and DP contents of 21 meat and milk products were measured. The highest TP and DP contents were found in processed and hard cheeses; the lowest, in milk and cottage cheese. TP and DP contents in sausages and cold cuts were lower than those in cheeses. Chicken, pork, beef, and rainbow trout contained similar amounts of TP, but slightly more variation was found in their DP contents. In conclusion, foods containing P

additives have a high DP content. The study confirms that cottage cheese and unenhanced meats are better choices than processed or hard cheeses, sausages, and cold cuts for CKD patients, based on their lower P-to-protein ratios and sodium contents. The results support previous findings of better P absorbability in foods of animal origin than in, for example, legumes.

Studies III and IV were controlled intervention studies in healthy young women. Each subject served as her own control, and the order of the study sessions was randomized. During each 24-h study session 24-h urine collections and four (Study III) or six (Study IV) blood samples were taken. Markers of mineral metabolism were measured, and analysis of variance with repeated measures was used to compare the study sessions. In Study III, acute effects of dietary P from three different food sources and a phosphate supplement on calcium and bone metabolism were investigated. Sixteen healthy young women participated in Study III. At the control session, calcium intake was ca. 250 mg and P intake ca. 500 mg. During the other four sessions P intake was about 1500 mg, 1000 mg of which was obtained from meat, cheese, whole grains, or a phosphate supplement, respectively. The foods served were exactly the same during the P sessions and the control session; only P sources varied. Only the phosphate supplement increased serum parathyroid hormone (S-PTH) concentration compared with the control session. Relative to the control session, meat increased markers of both bone formation and bone resorption. Cheese decreased S-PTH and bone resorption. These data suggest that the metabolic response was different for different foods.

The purpose of Study IV was to determine whether the effects of mono- and polyphosphate salts (MP and PP, respectively) used as additives differ on markers of Ca and P metabolism in young women. Fourteen healthy young women participated in the study. During each session the subjects received three doses of MP, PP, or a placebo with meals in randomized order. Both Pi salts provided 1500 mg P/day, and the diet during each session was identical. In both MP and PP sessions, serum phosphate, urinary phosphate, and S-PTH increased relative to the control session. PP decreased U-Ca more than did MP. The results suggest that PP binds Ca in the intestine better than does MP. Based on the S-Pi, U-Pi, and S-PTH results, both Pi salts are absorbed with equal efficiency. In the long run, increased S-PTH, caused by either an MP or PP salt, could have negative effects on bone metabolism.

In summary, the studies support the previous understanding of differences in P absorbability and mineral metabolism. This is relevant regarding the diets of CKD patients, but may also have a wider relevance if the significance of high P intake in the aetiology of cardiovascular diseases and osteoporosis is confirmed. Inorganic P used as additives seems to be absorbed more efficiently than P from other sources, and acutely affects bone metabolism negatively. Therefore, products containing P additives could be more harmful in a P-restricted diet. The effect of a P source also depends on the other nutrients that it contains. For example, in healhy subjects, hard cheese affected mineral

metabolism favourably due to its high calcium content. Based on P-to-protein ratios, the most suitable foods in a P-restricted diet are P-additive-free legumes and bakery products; among foods of animal origin, cottage cheese and P-additive-free meats are best.

List of original publications

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

I. **Karp H**, Ekholm P, Kemi V, Itkonen S, Hirvonen T, Närkki S, Lamberg-Allardt C. Differences among total and *in vitro* digestible phosphorus content of plant foods and beverages. J Ren Nutr 2012;22:416-422.

II. **Karp H**, Ekholm P, Kemi V, Hirvonen T, Lamberg-Allardt C. Differences among total and *in vitro* digestible phosphorus content of meat and milk products. J Ren Nutr 2012;22:344-349.

III. **Karp HJ**, Vaihia KP, Kärkkäinen MU, Niemistö MJ, Lamberg-Allardt CJ. Acute effects of different phosphorus sources on calcium and bone metabolism in young women: a whole-foods approach. Calcif Tissue Int 2007;80:251-258.

IV. **Karp HJ**, Kemi VE, Lamberg-Allardt CJ, Kärkkäinen MU. Mono- and polyphosphates have similar effects on calcium and phosphorus metabolism in healthy young women. Eur J Nutr 2013;52:991-996.

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In addition, some unpublished results are presented.

Contribution of the authors to Studies I-IV

I. HK, PE, VK, TH, and CLA planned the study. SN carried out the laboratory analysis. HK, PE, and SI analysed the data. HK wrote the manuscript with PE and TH, and the other co-authors critically reviewed the paper.
II. HK, PE, VK, TH, and CLA planned the study. The laboratory technician carried out the laboratory analysis. HK and PE analysed the data. HK wrote the manuscript with PE and TH, and the other co-authors critically reviewed the paper.
III. HJK planned the study with the co-authors. KPV recruited study subjects and conducted the experimental work. HJK did the laboratory and statistical analyses. HJK wrote the manuscript, and the co-authors critically reviewed the paper.
IV. HJK planned the study with the co-authors. HJK recruited study subjects and conducted the experimental work. HJK and VEK conducted the laboratory analysis with the laboratory technician. The author carried out the statistical analysis with MUK. HJK wrote the manuscript, and the co-authors critically reviewed the paper.

Abbreviations

$1,25(OH)_2D_3$	1,25 dihydroxyvitamin D, calcitriol
ANOVA	analysis of variance
AP	alkaline phosphatase
ATP	adenosine triphosphate
AUC	area under the curve
BALP	bone-specific alkaline phosphatase
BCE	bone collagen equivalent
BMD	bone mineral density
Ca	calcium
CKD	chronic kidney disease
CKD-MBD	chronic kidney disease mineral and bone disorder
Crea	creatinine
CTx	carboxy-terminal telopeptide of type I collagen
DMP	dentin matrix protein
DP	*in vitro* digestible phosphorus content
DPyr	deoxypyridinoline
DXA	dual x-ray absorptiometry
EFSA	European Food Safety Authority
ESRD	end-stage renal disease
FGF-23	fibroblast growth factor 23
FGF-7	fibroblast growth factor 7
GFR	glomerular filtration rate
iCa	ionized calcium
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-OES	inductively coupled plasma optical emission spectrometry
IL-10	interleukin 10
IL-6	interleukin 6
K	potassium
M-CSF	macrophage colony-stimulating factor
MEPE	matrix extracellular phosphoglycoprotein
MP	monophosphate
MRP	Maillard reaction product
Na	sodium
Npt	sodium-dependent phosphate transporter
NTx	amino-terminal telopeptide of type I collagen
OC	osteocalcin
Opg	osteoprotegerin
P	phosphorus
Phex	phosphate regulating gene with homologies to endopeptidases on the X chromosome

Pi	phosphate
PICP	carboxy-terminal propeptide
PINP	amino-terminal propeptide
Pit2	type III sodium-dependent phosphate transporter 2
PP	polyphosphate
PTH	parathyroid hormone
Pyr	pyridinoline
RANK	receptor activator of nuclear factor κB
RANKL	receptor activator of nuclear factor κB ligand
RDA	recommended daily allowance
sFRP-4	secreted frizzled related protein-4
TNF-α	tumour necrosis factor α
TP	total phosphorus content
TRACP	tartrate-resistant acid phosphatase
UL	tolerable upper intake level
VSMC	vascular smooth muscle cell

1 Introduction

Phosphorus (P) is abundantly found in several foods, the main ones being protein-rich foods such as dairy products, meat, grain, legumes, nuts, and seeds. In addition, P-containing additives are commonly used in the food industry in such foods as processed cheese and meat products, cola beverages, and bakery products. Differences in P absorbability among different food sources appear to exist (e.g. Kalantar-Zadeh *et al.* 2010). For instance, P from plant foods seems to be less well absorbed than from other sources, as P in plants is often in the form of phytic acid or its salts, which need to be hydrolysed for P to be available for absorption in the gut. By contrast, inorganic P used as a food additive may be nearly fully absorbed in the intestinal tract (Sullivan *et al.* 2007, Calvo 2000). Current data on food P content and P absorbability are scarce.

The recommended P intake for adults is 600-700 mg/d (Nordic Nutrition Recommendations 2013, Institute of Medicine 1997). P intake exceeds these recommendations 2- to 3-fold in several countries (Takeda *et al.* 2002, Gronowska-Senger & Kotańska 2004, Paturi *et al.* 2008, Welch *et al.* 2009). The use of P additives complicates the estimation of total P intake since food composition databases do not take into account the use of phosphate additives (Oenning *et al.* 1998, Sullivan *et al.* 2007). Hence, true P intakes may be higher than those found in studies.

P is needed in the body for maintaining cell structure, energy metabolism in generation of adenosine triphosphate (ATP), regulation of cellular signalling through phosphorylation of enzymes, maintaining acid-base homeostasis by urinary buffering, and bone mineralization as a part of hydroxyapatite. Phosphate homeostasis is regulated by parathyroid hormone (PTH), calcitriol, fibroblast growth factor 23 (FGF-23), and several other hormones (Berndt & Kumar 2009). During recent years discovery of FGF-23 and other phosphatonins, i.e. regulators affecting renal reabsorption of phosphate, has led to better understanding of phosphate metabolism and its endocrine linkage to bone, referred to as the bone-kidney axis. Even so, less is known about the mechanisms and regulation of intestinal phosphate (Pi) absorption than about phosphate handling by the kidney.

A high dietary P intake or high serum phosphate concentration has been associated with the aetiology or progression of certain diseases. A P-rich diet may have a negative effect on bone metabolism (Reiss *et al.* 1970, Brixen *et al.* 1992, Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006), which, in the long run, could lead to lower bone mineral density and osteoporosis. In chronic kidney disease, the ability to excrete phosphate is gradually lost. Therefore, to avoid complications related to hyperphosphatemia, treatment of Pi retention requires dietary P restriction with or without P binding medication. Early treatment of Pi retention or hyperphosphatemia may prevent or slow the development of the kidney disease. Recently, higher serum Pi levels even within the normal range have also been associated with increased risk of cardiovascular disease in healthy populations

with intact kidney function (Larsson *et al.* 2010, Foley *et al.* 2009, Foley *et al.* 2007, Dhingra *et al.* 2008).

Data on P content of foods are needed not only to be able to evaluate P intake of renal patients, but also to study P intake and its effects on health at the population level. Data on bioavailability of P from different food sources are also lacking. In this work, P content of foods, absorbability of P, and the effect of P on mineral metabolism are discussed.

2 Review of the literature

2.1 Phosphorus in foods

Even though most foods contain phosphorus (P), its main sources are protein-rich foods such as dairy products, meat, fish, eggs, legumes, nuts, and whole-grains (e.g. Calvo & Uribarri 2013). Total P per gram of protein is similar in plant and animal foods, ca. 20 mg/g protein in cheese, beef, lentils, and peas (Massey 2003). Regression equations to depict the association between protein and P intake have been developed (Boaz & Smetana 1996, Kalantar-Zadeh *et al.* 2010). In the study of Boaz and Smetana (1996), the following regression equation accounted for 84% of the variance in dietary P intake:

Dietary P (mg) = 128 mg P + (dietary protein in g) x 14 mg P / g protein.

In the study of Colman *et al.* (2005), the following regression equation accounted for 83% of the variation:

Dietary P (mg) = 78 mg P + (dietary protein) x 11.8 mg P / g protein.

It has been pointed out that these equations are not necessarily suitable for predicting individual P intakes (Agarwal & Agarwal 1997), but they illustrate the relationship between these two nutrients. The common use of P-containing additives further complicates this association (Kalantar-Zadeh *et al.* 2010).

2.1.1 Analysis of food phosphorus content

Foods are complex chemical systems in which multiple chemical reactions and interactions, as well as physical phenomena, are likely to occur (Molins 1991). This complicates nutrient analyses of foods. In P analysis, for example, meats contain phosphate hydrolytic enzymes capable of catalysing polyphosphate breakdown even at subzero temperatures, altering the composition of the added phosphates at rates that vary with product storage conditions. Furthermore, foods are able to bind, depolymerize, or otherwise affect phosphates to the extent of making them undetectable by the traditional analytical techniques used in pure phosphate solutions. Currently, several methods are used to analyse P content of foods.

In colorimetric methods, phosphates are converted to a coloured compound through a chemical reaction, which can then be detected, as the intensity of a colour is related to the amount of P in the sample. For example, in the validated method of the Nordic Committee on Food Analysis for P determination in food, the sample is dry-ashed to remove organic material (Nordic Committee on Food Analysis 1994). The acid-soluble inorganic residue is used for a colour reaction based on the formation of a blue complex between Pi and sodium molybdate in presence of ascorbic acid as a reducing agent. The intensity of the blue colour is measured spectrophotometrically. Cupisti *et al.* 2006, Benini *et al.* 2011,

and Cupisti *et al.* (2012) have used the molybdenum blue method in their P analyses. Moe *et al.* (2011) measured "photometrically" the P contents of the diets in their crossover trial, but did not describe their method more specifically.

The molybdenum blue method has many applications (Molins 1991). For instance, several reducing agents have been used successfully to develop the molybdenum blue colour for analysing P in diverse foods. Another colorimetric method uses vanadomolybdate reagent, which produces a yellow compound when reacting with phosphates (Olsen & Sommers 1982). The official method of AOAC International 965.17 for P analysis (Phosphorus in Animal Feed and Pet Food) is similar to this method. The P contents of several grain products in the Finnish food composition database Fineli® (National Institute for Health and Welfare 2011) are based on the findings of Varo *et al.* (1980), who used a vanadomolybdate method in their analyses (Saari & Paaso 1980).

AOAC Official Method 964.06 (Phosphorus in Animal Feed) is also used in food analyses. In this titrimetric method, molybdate solution and sodium hydroxide are used as reagents. Phenolphtalein is added to produce a pink colour, which is titrated with standard acid. Another official method for determining P in flour (AOAC Official Method 948.09 Phosphorus in Flour) has been published. In this method, either magnesium nitrate solution or molybdate solution can be used as reagents. Similarly to the former method, the pink colour is titrated with standard acid.

P content of a food can also be measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES), also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES). ICP-OES is a type of emission spectroscopy using the inductively coupled plasma to transform atoms of the sample into the excited state and also to ionize them. The excited atoms and ions emit electromagnetic radiation at the wavelengths characteristic of a particular element, and the intensity of this emission is indicative of the concentration of the element in the sample. AOAC Official Method 984.27 (Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc in Infant Formula) is based on ICP-OES. Sullivan *et al.* (2007), Sherman & Mehta (2009a), Sherman & Mehta (2009b), and León *et al.* (2013) have used ICP-OES in their analyses of meat P content. An X-ray fluorescence method for analysing P in meat products has also been published (Jastrzębska *et al.* 2010).

Recently, methods to detect different fractions of P in foods have been developed (Benini *et al.* 2011, Cupisti *et al.* 2012, Itkonen *et al.* 2012). Itkonen *et al.* (2012) tested a method for analysing digestible P content of foods, which is expected to represent the amount of bioavailable P. This method is based on enzymatic assay of insoluble and soluble dietary fibre (Asp *et al.* 1983). The proteins and starch of the sample are degraded with alimentary enzymes and the mineral content in the remaining fibre is analysed. Ekholm *et al.* (2000, 2003) developed the method for the analysis of soluble minerals. Digestible P was analysed using an inductively coupled plasma-mass spectrometry (ICP-MS) (Itkonen *et al.*

2012). The authors concluded that the new method for digestible P analysis is reliable, but requires validation by *in vivo* tests.

Benini *et al.* (2011) determined total and soluble P contents of three types of meat products prepared both with and without P additives. Soluble Pi ions were extracted with a slight modification in a procedure described previously by Jastrzębska *et al.* (2008). Both total and soluble P were analyzed by a spectrophotometrical molybdenum blue method (Benini *et al.* 2011). Subsequently, the same authors described a novel method for analysing three different P subtypes of ham (inorganic P including added preservatives and natural P derived from phospholipids and phosphoproteins) separately (Cupisti *et al.* 2012). In this method, inorganic P was measured similarly to the soluble P analysis previously reported (Benini *et al.* 2011). For the analysis of P in phospholipids, lipid extraction was performed, and P content of the lipid extract was measured (Cupisti *et al.* 2012). P content of phosphoproteins was measured after precipitation of trichloroacetic acid. The authors conclude that their novel method is feasible and can accurately detect the added P additives in meat products. They also believe the method can be extended to all common foods of both animal and plant origin, also including determination of P from phytate in plant products (Cupisti *et al.* 2012).

2.1.2 Phosphorus sources

2.1.2.1 Natural sources

In cow milk, the concentration of P is ca. 95 mg/100 g (Gaucheron 2011). The P content of human milk is much lower, 10 mg/100 g according to the Finnish food composition database Fineli® (National Institute for Health and Welfare 2011). The highest P contents in dairy foods are found in hard and processed cheeses (500-800 mg/100 g), the lowest in milk and fresh cheeses (e.g. Gaucheron 2011). The P contents of selected dairy products are shown in Appendix I, Table 1. The chemical composition of P in milk is complex. Milk contains both organic and inorganic phosphates. Organic phosphate is phosphate bound to organic molecules of milk such as casein, phospholipids, RNA, DNA, nucleosides, nucleotides, and sugar phosphate. Phosphates associated with casein molecules are in the micellar phase, and phosphates with other organic molecules of milk are in the aqueous phase. Inorganic phosphates are distributed between the aqueous and micellar phases. In casein micelles, both organic and inorganic phosphates are associated with calcium (Ca). These associations between Ca and phosphate form granules known as nanoclusters. During preparation of dairy products inorganic phosphates can be transferred to the aqueous phase (Gaucheron 2011). Hence, part of P is lost in whey during cheese making. This partly explains the different amounts of P in different dairy products.

Phosphorus in muscle protein foods occurs as phosphate bound to amino acid side chains, but also as phospholipids and nucleotides (Massey 2003). The P contents of raw (lean and boneless) meats are similar, with the exception of reindeer, the P content of which is higher than in other meats based on Fineli® (Appendix I, Table 2). In liver and kidney, the P content is higher than in muscle meats; on average 390 and 260 mg, respectively (National Institute for Health and Welfare 2011). In eggs, almost all P is in the egg yolk (Kalantar-Zadeh *et al.* 2010).

The P content of fishes varies among species. The P contents of salmon and rainbow trout, which are commonly consumed in Finland, are 240 and 260 mg/100 g, respectively (National Institute for Health and Welfare 2011). Among raw filleted marine fish species, the lowest P content has been found in broomtail grouper (103 mg/100 g) and the highest in pompano (287 mg/100 g) (Castro-González & Miranda-Becerra 2010). Fatty fishes seem to have higher P content than lean fishes. Furthermore, because fish bones consist of calcium phosphate, their P content is high. Therefore, small fishes consumed with their bones, such as canned sardines (Gastro-González *et al.* 2010), have relatively high P content (279-304 mg/100 g). Variation also in the P content of molluscs has been found (Castro-González & Miranda-Becerra 2010).

When considering both meats and fishes, cooking is likely to alter their P content, probably depending on the specific cooking method (Sherman & Mehta 2009a). Boiling foods may reduce P retention as compared with raw samples (Cupisti *et al.* 2006). Also soaking in water (before cooking) reduces the P content of a food (Jones 2001). Although data on the effect of other cooking methods on the P content of foods do not exist, the P content of cooked meat or fish may often be higher than that of raw food (National Institute for Health and Welfare 2011), as the water content of cooked meat is lower than that of raw meat.

Among plant foods, most fruits and vegetables contain only small amounts of P (Kalantar-Zadeh *et al.* 2010). By contrast, organic P is found abundantly in seeds, nuts, and legumes, but also in cereals. Chocolate, which is made from the seed of the cacao tree flower, is also a P-rich food, the highest amounts being found in milk chocolate (Noori *et al.* 2010). P in plants is often in the form of phytic acid (myoinositol hexaphosphate), its free salt phytate, or its Ca/magnesium (Mg) salt phytin (Plaami 1997). In seeds, 60-90% of the total P occurs as phytic P (Ravindran *et al.* 1994, see Plaami 1997). The proportion of phytate P is much lower (10-25%) in tubers (Ravindran *et al*. 1994). In legumes, phytate content differs among species and forms, with broad beans having the highest and lentils the lowest content (Elhardallou & Walker 1994). In cereals, phytate is mostly located in the aleurone layer and the germ, while the endosperm is almost free of phytate (O'Dell *et al.* 1972). Hence, whole-grain products have a higher P content than hulled products. Varying degrees of phytates are also present in fruits and vegetables. Leafy vegetables seem to be an exception, as they are practically devoid of phytate.

The bioavailability of P from phytic acid and its salts is lower than in P from foods of animal origin (see Section 2.1.3). Furthermore, food composition databases may not be accurate in their plant-food P contents, as Moe *et al.* (2011) found a 33% lower P content in a laboratory-analysed grain- and soy-based diet than that predicted from the database, while the P content of a meat-based diet was predicted correctly by the database. P contents of selected plant foods are shown in Appendix I, Table 3.

2.1.2.2 Phosphorus-containing food additives

Several P-containing food additives are used by the food industry (Kalantar-Zadeh *et al.* 2010, Uribarri 2007). In the European Union, all additives in packed foods are required to be designated by their category, indicating the purpose of the additive followed by either the specific name of the additive or an E code for permitted additives (European Council 2000). In other countries, e.g. in the United States, identification of products with P-containing additives may be less consistent (e.g. Sullivan *et al.* 2007). The P-containing additives with their E codes are shown in Appendix II.

P-containing additives are used for a number of reasons, enabling food products to achieve, for example, better texture, taste, emulsification, acidification, leavening, anti-caking, moisture binding, antimicrobial action, colour stability, iron binding, buffering, and freeze-thaw stability (Calvo & Uribarri 2013). The use of P-containing additives is very common in the meat industry, where they are used in both raw and cooked meats, sausages, and other restructured meats. In dairy products, P additives are most commonly found in processed cheeses, in which phosphates function as melting salts. Cola and some other soft drinks contain varying amounts of phosphoric acid to affect the colour, texture, or taste of these beverages (Gutekunst 2010, Ritz *et al.* 2012). Another reason speculated for using phosphoric acid in soft drinks instead of citric or tartaric acid is its cheaper price and ability to produce greater sourness (Uribarri 2007).

Bakery products containing sodium phosphate as a baking powder are also a common P additive-containing group of foods (Uribarri 2007). In addition, as phosphates prevent agglomeration of food powders, they are used in different types of instant products such as powdered coffee and pudding (Ritz *et al.* 2012). Frozen fish products are commonly rinsed before freezing in a solution of 12.5% sodium tripolyphosphate to prevent loss of proteins during thawing (Uribarri 2007). Frozen foods (72%) were the most common P additive-containing food group in a study reviewing nearly 2400 best-selling branded grocery products in northeast Ohio for P additives (León *et al.* 2013). Other food groups commonly containing P additives were dry food mixes (70%), packaged meat (65%), bread and bakery products (57%), soup (54%), and yogurt (51%).

Based on analyses on several meat products, P additives substantially increase the P content of the product relative to a similar product prepared without P-containing additives

(Sullivan *et al.* 2007, Sherman & Mehta 2009a, Sherman & Mehta 2009b, Benini *et al.* 2011, Cupisti *et al.* 2012). The difference in P content to a product prepared without additives may be up to twofold (Sherman & Mehta 2009a). Furthermore, food composition databases may underestimate P contents of foods (Sullivan *et al.* 2007). The variation in the use of P additives in similar kinds of products complicates the estimation of P content of a product or a diet. Table 1 shows the P content of cooked ham prepared with or without P additives based on different sources. The method described by Cupisti *et al.* (2012) to quantify different subtypes of P in meat products could help to estimate P intake originating from additives. So far, analysed data on the effect of P additives on the P content of a single product are not available in food groups other than meats. However, León *et al.* (2013) measured P contents over four days in sample meals created from local best-selling branded grocery products with and without P additives. Sample meals comprising mostly P additive-containing foods had >700 mg more P/day than meals consisting of only additive-free foods. P additive-free meals were, however, more expensive than P additive-containing meals.

Table 1. *Phosphorus content of cooked ham prepared with and without additives based on different sources.*

Phosphorus content in cooked ham (mg /100 g)		Source
Without additives	With additives	
194±7	296±47	Benini *et al.* 2010
185±17	270±46	Cupisti *et al.* 2012
*200 (fat content 4.5%)		Fineli®
*160 (fat content 3.1%)		Fineli®
*239 (water added, boiled)		U.S. Dept of Agriculture (2012)

*Use of P additives not mentioned

2.1.3 Bioavailability of phosphorus

With regard to nutrients, bioavailability usually designates the quantity or fraction of the ingested dose absorbed (Heaney 2001). However, bioavailability may also include transport of nutrients to organs and cells, where they finally fulfill their physiological function (Schlemmer 1995). Schlemmer (1995) notes that bioavailability is not merely a question of a nutrient's features that allow the nutrient to be utilized, but is also affected by factors of different individuals such as requirement of the nutrient, age, sex, etc. For simplicity, bioavailability here is considered similarly to Heaney's above-mentioned definition.

Several *in vivo* methods are used to estimate bioavailability (Heaney 2001). The classic balance method means measuring the difference between the ingested amount of a nutrient

and the amount found in faeces. This method is, however, imprecise, time-consuming, laborious, and expensive. If combined with intestinal lavage, it is more accurate and simpler to perform. Measurement of the serum concentration of a nutrient after ingestion does not yield absolute bioavailability values, but can be used in comparisons of two or more preparations. The problem regarding this method is the homeostatic regulation of serum concentrations, which may actively damp the absorptive rise. According to Heaney (2001), using either radioactive or stable tracers in evaluating bioavailability would appear to be optimal for most nutrients. The tracer method is sensitive, but its limitation is that it requires that the source can be intrinsically labelled. *In vitro* digestion models are methods in which enzymatic degradation of food is simulated outside the body (Hur *et al.* 2011). Compared with *in vivo* methods, they are more rapid and economic, but less accurate.

Of natural P sources in a mixed diet, 60-80% is absorbed in the intestine, as measured by a balance method combined with intestinal lavage (Ramirez *et al.* 1986). The absorption rate of P from an individual food may, however, differ substantially depending on the form of P found in the food and other nutrients of the meal (e.g. Uribarri 2007, Kalantar-Zadeh *et al.* 2010). The physiology of P absorption is discussed in Section 2.3.2.

In milk, P absorbability differs among different fractions (Uribarri & Calvo 2003). The P in casein micelles is of lower bioavailability. On the other hand, dairy foods contain abundant Ca, which complexes with phosphate in the intestine, thereby reducing its absorption (Heaney & Nordin 2002). Ca supplements are commonly utilized in the treatment of hyperphosphatemia in chronic kidney disease to bind dietary P (Nolan & Cunibi 2003). Hence, theoretically, P from dairy foods could have a relatively low bioavailability. However, based on increased serum phosphate levels after both cheese and milk ingestion in a controlled human study, P from dairy foods seems to be absorbed efficiently (Kärkkäinen *et al.* 1997). In a rat study, P from casein affected phosphate metabolism more profoundly than the same amount of P from grains, implying relatively good P bioavailability from the former (Moe *et al.* 2009). In addition, the Institute of Medicine (1997) states that there is "no significant interference with phosphorus absorption by calcium at intakes within the typical adult range". P absorption is more efficient from human milk than from cow milk (Williams *et al.* 1970).

Also, data on P bioavailability from meat are scarce, even though generally P from meat is considered to be well absorbed (Uribarri 2007). Based on a balance study in healthy young men, P from meat is absorbed efficiently (Schuette & Linkswiler 1982). From hydroxyapatite, P seems to be well absorbed, based on an experimental study in healthy volunteers fed bone meal powder (Tsuboi *et al.* 2000). However, P in bone meal powder (hydroxyapatite) is in a different form than in meat. The results of Tsuboi *et al.* (2000) could be extrapolated to P absorbability from bony fish as bones from other animals are not normally consumed.

In plant foods, phytates need to be hydrolysed for P to be available for absorption. Phytate hydrolysation may occur enzymatically by phytase enzyme or non-enzymatically via acid, high temperature, or pressure (Plaami 1997). Phytase enzymes are found in plants, microbes, and some animal tissues. In humans, phytase activity is very low in the gut (Iqbal *et al.* 1994), but processing of foods by soaking, germinating, or fermenting reduces phytate content by activating intrinsic phytase (Beal & Mehta 1985). Boiling and steaming also degrade phytates (Plaami 1997). During bread-making phytic acid content of the bread is decreased due to both the activation of phytases present in the flour and high temperature. Baker's yeast has phytase activity, and a larger amount of phytic acid is degraded in breads leavened with yeast than in breads prepared without it (Türk & Sandberg 1992). However, also souring degrades phytic acid based on a recent *in vitro* study, in which non-soured cereals contained lower percentages of digestible P than soured cereals (Itkonen *et al.* 2012). Furthermore, several other factors contribute to phytate degradation in doughs such as particle size of flour, pH, temperature, water content, and fermentation time (Plaami 1997). Also, phytase activity and its heat resistance may differ among different grains (McCance & Widdowson 1944). In any case, a larger amount of P is likely to be bioavailable from cooked or otherwise processed plant foods than from raw or unprocessed foods.

The bioavailability of P from phytic acid-containing plant foods has mostly been studied in farm animals. The only human study on the effect of phytic acid on P absorption is in ileostomic patients, in whom phytic acid reduced absorption of P (Kivistö *et al.* 1986). Similar results were found more recently, as a vegetarian diet caused lower serum Pi concentration than a meat-based diet in a crossover trial in chronic kidney disease patients, implying thus lower bioavailability for vegetarian than for meat P sources (Moe *et al.* 2011). Adding phytase to the feed of pigs and chickens has been shown to improve P bioavailability (Simons & Versteegh 1990, Sands *et al.* 2001, Liu *et al.* 2007). Compared with conventional feed, P absorbability from low-phytate soybean (Dilger & Areola 2006) and barley (Veum *et al.* 2002) has been improved in chicken and in pigs, respectively.

P additives in foods are inorganic compounds, which are not protein-bound (Kalantar-Zadeh *et al.* 2010). For this reason, it has been estimated that inorganic P compounds more readily dissociate in the gut, and more than 90% of inorganic P may be absorbed in the intestinal tract (Calvo 2000, Sullivan *et al.* 2007). Hence, P additives not only increase the P content of the food, but also the proportion of absorbable P compared with foods containing only natural P. The bioavailability of P additives may differ, as polyphosphates have caused a more severe nephrocalcinosis in rats than monophosphates (Matsuzaki *et al.* 1999, Matsuzaki *et al.* 2001). However, monophosphates may increase PTH secretion more than polyphosphates (Zemel & Linkswiler 1981).

Contrary to phytic acid-containing foods, processing might also impair the bioavailability of P, as consumption of a Maillard reaction product (MRP) -rich diet had a negative influence on dietary P absorption, compared with a diet low in MRPs (Delgado-Andrade *et*

al. 2011). Processing of foods with high protein and carbohydrate and/or fat content favours development of the Maillard reaction and formation of browning products, especially by frying, roasting, grilling, baking, and reheating. Thus, MRPs are formed especially during the manufacturing of snacks and fast foods. For this reason, increased snacking and fast food consumption are thought to impair bioavailability of P and other minerals (Delgado-Andrade *et al.* 2011).

Of other nutritional interactions affecting P bioavailability, pharmacological doses of nicotinamide (Eto *et al.* 2005), and aluminium (Rutherford *et al.* 1973) prevent P absorption in the intestine. Polyphenols of tea seem not to affect P absorption based on a rat study on white tea extract (Pérez-Llamas *et al.* 2011). Taken together, P bioavailability depends on the predominant food groups, the degree of processing of the foods, and the amount of P additives in the foods consumed. Still, P is absorbed more efficiently than several other minerals.

2.2 Dietary requirements, guidelines, and intake of phosphorus

The recommended intake of P in adults set by the Nordic Nutrition Recommendations is 600 mg/d (Nordic Nutrition Recommendations 2013). The recommended daily allowance (RDA) for adults aged ≥19 years by the Institute of Medicine (1997) is 700 mg/d. The RDA for children aged 9-18 years is substantially higher, 1250 mg/d, due to an estimated additional need during growth. In Table 2, the recommended intakes of P for different age groups according to the Nordic Nutrition Recommendations and the Institute of Medicine are compared.

Table 2. *Recommended intake of phosphorus by the Nordic Nutrition Recommendations (2013) and the dietary reference intakes of the Institute of Medicine (1997).*

Nordic Nutrition Recommendations 2013		**Recommended daily allowance**	
Age/life stage	Recommended P intake (mg/d)	Age/life stage	Recommended P intake (mg/d)
<6 mo	-	0-6 mo	100*
6-11 mo	420	7-12 mo	275*
1-5 y	470	1-3 y	460
6-9 y	540	4-8 y	500
10-17 y	700	9-18 y	1250
≥18 y	600	≥19 y	700
Pregnancy	700	Pregnancy	700
Lactation	900	Lactation	700

*Adequate intake

The dietary recommendations for P by the Institute of Medicine (1997) have been set using balance studies and serum inorganic P (S-Pi) concentrations. The level required to maintain S-Pi within an optimal range has been considered an indicator of P nutrition. For infants and children, the recommendations are based on estimates of the need for P during growth.

Some variation exists in the highest acceptable intake of P among different recommendations. The tolerable upper intake level (UL) of P set for children aged 1-8 years and adults >70 years is 3 g/d, whereas UL for individuals aged 9-70 years is higher, 4 g/d (Institute of Medicine 1997). For pregnant women, the UL is 3.5 g/d. The European Food Safety Authority (EFSA) has not established an upper intake level for P, but states that healthy individuals can tolerate P intakes of "up to at least 3000 mg/d without adverse systemic effects" (EFSA 2005). According to the Nordic Nutrition Recommendations (2013), the tolerable upper intake level has to be re-evaluated during the coming years due to recent observations on adverse effects of P.

Compared with the recommended intakes, the average P intake of adults is generous based on data from several countries (Takeda *et al.* 2002, Gronowska-Senger & Kotańska 2004, Paturi *et al.* 2008, Welch *et al.* 2009). In Finland, the mean intake of P among 25- to 64-year-olds was 1326 mg/d in women and 1778 mg/d in men (Paturi *et al*. 2008). In the EPIC study, geographical variation in P intakes among 35- to 74-year-old adults in 10 European countries was reported (Welch *et al.* 2009). In men, the lowest intake (1425 mg/d) was found in the United Kingdom in a cohort consisting predominantly of vegetarians, and the highest (2070 mg/d) in Greece. In women, P intakes ranged from 1089 mg/d in Ragusa, Italy to 1478 mg/d in Aarhus, Denmark. In the USA, P intakes in

the 2005-2006 National Health and Nutrition Survey (NHANES) were 1727 mg and 1197 mg in men and women, respectively (Calvo & Uribarri 2013). In the 2009-2010 survey, the intakes were slightly higher, which may be due to updates in the P content of food composition data (Calvo & Uribarri 2013).

P intake may be adequate despite lack of several other nutrients in the diet, as observed in Turkish adolescents (Garipagaoglu *et al.* 2008). On the other hand, relatively low P intakes (649 mg/day) have been found in Japanese 20- to 23-year-old female college students together with inadequate Ca, protein, and potassium (K) intakes (Ueno *et al.* 2005). Heaney (2004) has raised the question of whether P intakes of older women taking Ca supplements are adequate, considering the low P intakes (10-15%) of women in this age group, and the P-binding capacity of Ca supplements. Furthermore, individuals with a lower socio-economic status and a lower income have a higher serum Pi, possibly indicating higher P intake due to fast food and preprepared meal consumption (Gutiérrez *et al.* 2010).

The use of P additives complicates estimation of total P intake since current food composition databases do not take into account the use of phosphate additives (Oenning *et al.* 1998, Sullivan *et al.* 2007). The true average intakes in Western countries may therefore be substantially higher than the current data show. Recently, in the USA, nutrient contents have been revised for chicken breasts and pork to reflect the use of P additives (Calvo & Uribarri 2013), but when regarding all P additive-containing foods, the need for more extensive updates exists. It has been estimated that in 1990 P intake from additives was about 500 mg/day in the USA (Calvo & Park 1996). However, already in the 1970s, Bell *et al.* (1977) calculated that depending on the food choices, P intake from additives could rise to 1 g/day. In Europe, Benini *et al.* (2010) have estimated that P additives increase the daily P intake by at least 100-300 mg/day in hemodialysis patients. In a recent study comparing the P content of top-selling foods with and without P additives, meals comprising mostly phosphorus additive-containing foods had 736 mg more P per day compared with meals consisting of only additive-free foods (León *et al.* 2013).

2.3 Metabolism of phosphorus

2.3.1 Function and distribution

P is needed in multiple biological processes. P-containing compounds are needed in the cell structure for maintenance of membrane integrity and nucleic acids, energy metabolism in generation of ATP, regulation of cellular signalling through phosphorylation of enzymes, maintenance of acid-base homeostasis by urinary buffering, and bone mineralization as a part of hydroxyapatite. In biological systems, P is present as phosphate,

i.e. $H_2PO_4^-$ and HPO_4^{2-}. It is the most abundant anion in the human body, comprising ca. 1% of total body weight (Penido & Alon 2012).

Most (85%) of the P in body content is in the bone and teeth, 14% in soft tissues, and only 1% in the extracellular space (Penido & Alon 2012). In the extracellular fluid, one-third of the P content is complexed to Na, Ca, and Mg, one-tenth is bound to proteins, and the rest is present as inorganic Pi. The serum Pi (S-Pi) concentration is determined by the balance between intestinal absorption of Pi from the diet (16 mg/kg/day), storage of Pi in the skeleton (3 mg/kg/day), and excretion of Pi through the urine (13 mg/kg/day) (Berndt & Kumar 2009). Central endocrine factors affecting these processes are parathyroid hormone (PTH), calcitriol ($1,25(OH)_2D_3$), and phosphatonins such as fibroblast growth factor-23 (FGF-23). In the following sections, P homeostasis and its regulation are reviewed in more detail.

Measuring the S-Pi content is the gold standard to estimate the overall Pi status of the body (Osuka & Razzaque 2012). However, as S-Pi is regulated homeostatically, its abnormal concentrations are often a sign of a metabolic disturbance caused by, for instance, an illness. The normal range for S-Pi concentration is 0.8-1.5 mmol/l in adults. Infants have the highest physiological (1.5-2.65 mmol/l) S-Pi concentration due to rapid bone growth and soft tissue buid-up. In addition to variation with age, S-Pi concentration varies over the course of the day, with a decrease to a nadir just before noon, an increase to a plateau in late afternoon, and a small further increase to a peak shortly after midnight (Portale *et al.* 1987). This diurnal variation may be substantially blunted in advanced chronic kidney disease patients (Moe *et al.* 2011). Also P intake from meals during the day strongly affects S-Pi concentrations

2.3.2 Intestinal absorption

Dietary P is absorbed as inorganic Pi throughout the small intestine. The duodenum seems to play an important role in the acute response to a high-P diet, whereas the jejunum is more significant in the chronic adaptation to a low-P diet (Giral *et al.* 2009). Two distinct mechanisms exist in Pi absorption: a passive paracellular pathway and active transport through the sodium-dependent phosphate co-transporters situated at the intestinal brush border membrane (Sabbagh *et al.* 2011). The paracellular transport of Pi by diffusion is largely dependent on the concentration of P present in the intestine. The paracellular pathway has been considered the major route of Pi entry (Kayne *et al.* 1993). However, based on recent studies on rats, at least in postprandial conditions, active transport has been shown to contribute up to 50% of the total P uptake (Sabbagh *et al.* 2009). Still, the importance of the paracellular route is supported by the early finding that Pi absorption remains relatively effective even at extremely low plasma $1,25(OH)_2D_3$ concentrations (Wiltz *et al.* 1979).

Several sodium-dependent Pi transporters exist (Murer *et al.* 2004). Type IIb sodium-dependent Pi transporter (Npt2b) is the major handler of P in the intestine; >90% of active intestinal P transport occurs through Npt2b (Sabbagh *et al.* 2011). Npt2b has a preference for divalent Pi (HPO_4^{2-}), and it transports Pi with a stoichiometry of 3 Na ions: 1 phosphate (Marks *et al.* 2010). Energy for the Pi transport across the brush border membrane is provided by the Na gradient (outside>inside) maintained by Na^+K^+ATPase at the basolateral membrane (Carpenter 2010). Inside the enterocyte, restricted channels, such as microtubules, carry Pi from the apical pole to the basolateral pole of the cell. Very little is known about the processes involved in the exit of Pi across the basolateral membrane into the circulation (Marks *et al.* 2010). The intestinal absorption of Pi is depicted in Figure 1.

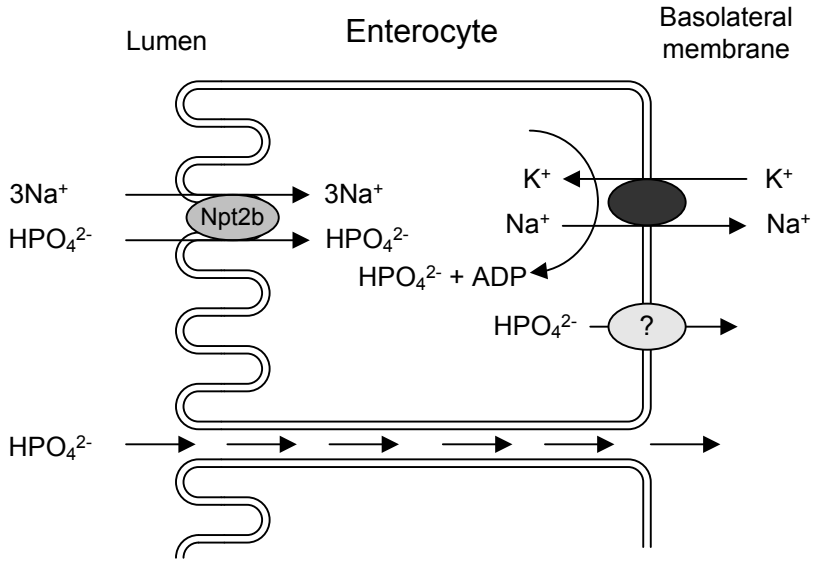

Figure 1 *P absorption in the enterocyte (modified from Carpenter 2010). Active transport occurs through Npt2b, and passive diffusion takes place paracellularly. Na^+K^+ATPase maintains the sodium gradient required for active transport. Exit of Pi from the enterocyte across the basolateral membrane is poorly understood.*

The amount of Npt2b is increased by $1,25(OH)_2D_3$ and dietary Pi restriction (Hattenhauer *et al.* 1999). PTH stimulates Npt2b indirectly, through its stimulatory effect on $1,25(OH)_2D_3$ synthesis (Penido & Alon 2012). Among the phosphatonins, which are a group of phosphaturic proteins regulating Pi homeostasis, matrix extracellular phosphoglycoprotein (MEPE) seems to be the only one affecting intestinal Pi absorption directly (Marks *et al.* 2008). In mice, MEPE inhibits Pi transport in the jejunum, but not in the duodenum. Other factors affecting intestinal Pi absorption include glucocorticoids, oestrogens, and metabolic acidosis (Penido & Alon 2012). Furthermore, there is an age-related decline in Pi absorption that is correlated with decreased gene and protein expression of Npt2b (Xu *et al.* 2002).

Relatively recently, it has been discovered that the amount of intestinal Pi can rapidly modulate renal Pi reabsorption (Berndt *et al.* 2007). In fact, when intestinal Npt2b is downregulated, there is an increase in renal sodium-dependent Pi transporter 2a (Npt2a), increasing Pi reabsorption (Sabbagh *et al.* 2009). It has been proposed that small intestinal mucosa secretes a thus far unknown substance, "intestinal phosphatonin", which regulates this renal-gastrointestinal axis. MEPE has been postulated to be this substance (e.g. Marks *et al.* 2010). The findings on the signalling axis between the intestine and the kidney suggest that intestinal Pi absorption plays a more significant role in Pi homeostasis than previously recognized. Still, less is known about the mechanisms and regulation of intestinal Pi absorption than about phosphate handling by the kidney. For example, it is not known whether Npt2b dominates only under fasting or low Pi conditions and whether Na-independent transport has a more important role in overall Pi absorption. Furthermore, regional-specific adaptations of intestinal Pi transport to acute and chronic Pi loads may exist. Even so, it seems that the main determinants of how much P is absorbed in the intestine are the P intake, its bioavailability, and the presence of either natural or pharmacologic P binders (Uribarri 2007).

2.3.3 Excretion

Renal excretion is the most important regulator of Pi homeostasis. Most (80%) of the Pi in the primary urine is reabsorbed in the proximal tubules. In the tubular cells, there is no paracellular transport of Pi, but Pi is absorbed by Npts (Biber *et al.* 2009). Npt2a has been localized throughout the proximal tubule (Custer *et al.* 1994) and is responsible for ~70% of Pi reabsorption in mice (Beck *et al.* 1998). Like Npt2b, Npt2a preferentially carries divalent Pi (HPO_4^{2-}) and transports it with a stoichiometry of 3 Na ions: 1 phosphate (Marks *et al.* 2010). PTH, FGF-23, and dietary Pi are considered to be the most important regulators of Npt2a protein levels. Expression of Npt2a is reduced within minutes to PTH, and within 2 h in response to a change in dietary Pi load (Penido & Alon 2012). Furthermore, a low pH caused by metabolic acidosis directly inhibits Npt2a (Nowik *et al.* 2008).

Sodium-dependent Pi transporter 2c (Npt2c) is present only at one segment (S1) of the proximal tubule (Nowik *et al.* 2008), and accounts for ~30% of Pi reabsorption in mice (Tenenhouse *et al.* 2003). In humans, however, the contribution of the Npt2c to renal Pi reabsorption and Pi homeostasis seems to be more important than in mice Biber *et al.* 2009). Npt2c shows a preference for divalent Pi (HPO_4^{2-}), with a stoichiometry of 2 Na ions: 1 phosphate (Marks *et al.* 2010). Dietary Pi decreases expression of Npt2c, but the response is slower than that of Npt2a (Segawa *et al.* 2005). FGF-23 (Segawa *et al.* 2003) and magnesium (Thumfart *et al.* 2008) increase Npt2c expression, whereas the role of PTH in Npt2c regulation is less clear (Marks *et al.* 2010).

In the absence of type II transporters, there is still residual renal Pi reabsorption (Segawa *et al.* 2009), suggesting a role for other Pi transporters. Of type III transporters, Pit2 has been localized in the kidney in the S1 segment of the proximal tubule under normal Pi intake, while dietary Pi restriction induces expression of Pit2 protein in all segments of the proximal tubule (Breusegem *et al.* 2009). Pit2 preferentially carries monovalent Pi ($H_2PO_4^-$), and it transports it with a stoichiometry of 2 Na ions: 1 phosphate (Marks *et al.* 2010). Similar to Npt2c, Pit2 adapts to changes in dietary Pi content more slowly than Npt2a. Furthermore, dietary K deficiency and FGF-23 decrease and metabolic acidosis increases renal Pit2 protein levels. Still, the role and importance of type III transporters in renal Pi handling under different conditions remain obscure. The tubular reabsorption of Pi is depicted in Figure 2.

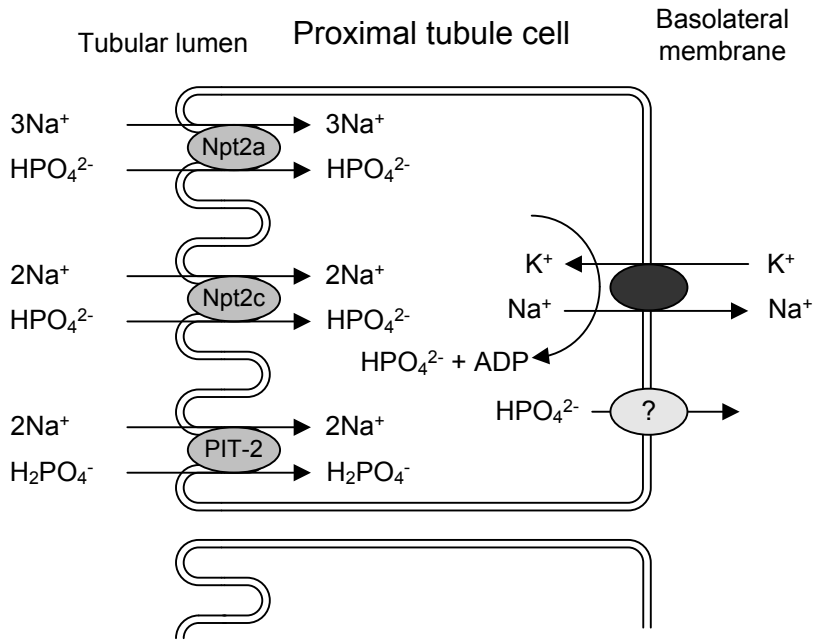

Figure 2 *Pi reabsorption in the proximal tubule cell (modified from Carpenter 2010). Three different Pi transporters (Npt2a, Npt2c, Pit-2) carry Pi in the cell.*

Several other hormonal factors affect renal Pi excretion in addition to PTH and FGF-23. Insulin, growth hormone, and thyroid hormone increase Pi reabsorption by increasing Na-Pi cotransport (Penido & Alon 2012). Calcitonin, glucocorticoids, and phosphatonins decrease renal reabsorption. Factors affecting Pi absorption or reabsorption are summarized in Table 3.

Table 3. *Factors influencing phosphate absorption or reabsorption in the intestine and kidney. Adapted from Berndt and Kumar (2009).*

Intestine	Kidney
Factors that increase Pi absorption 1. Reduced dietary intake of P 2. Elevated serum 1,25-dihydroxyvitamin D	*Factors that increase Pi reabsorption* 1. Phosphate depletion 2. Parathyroidectomy 3. 1,25-dihydroxyvitamin D 4. Volume contraction 5. Hypocalcemia 6. Hypocapnia
Factors that reduce Pi absorption 1. Reduced serum 1,25-dihydroxyvitamin D 2. Elevated concentrations of Ca salts in intestinal lumen 3. MEPE	*Factors that reduce Pi reabsorption* 1. Pi loading 2. PTH and cyclic AMP 3. Volume expansion 4. Hypercalcemia 5. Carbonic anhydrase inhibitors 6. Dopamine 7. Glucose and alanine 8. Acid-base disturbances 9. Increased bicarbonate 10. Hypercapnia 11. Metabolic inhibitors 12. Arsenate 13. FGF-23 14. sFRP-4 15. MEPE 16. FGF-7

2.3.4 Hormones involved in phosphate homeostasis

2.3.4.1 Parathyroid hormone

The primary function of PTH is to tightly regulate serum Ca concentration. Hypocalcemia stimulates parathyroid glands to produce and release the hormone. PTH increases expression of 25(OH)D 1α-hydroxylase in the kidney, resulting in increased production of calcitriol, and consequently, increased Ca and Pi absorption in the intestine. The increase in Pi absorption is mediated by the increase in Npt2b (see Section 2.3.2). PTH also enhances renal Ca reabsorption and reduces renal Pi reabsorption by reducing expression of Npt2a and possibly Npt2c (see Section 2.3.3). In bone, PTH increases the release of Ca and Pi into the extracellular fluid by stimulating osteoclastic bone resorption. As a result, the combined effects of efflux of Ca from bone, conservation of Ca by the kidney, and increased dietary absorption of Ca restores serum Ca to normal. The increased Pi efflux from bone and influx from the gastrointestinal tract is balanced by PTH effects to decrease renal tubular Pi reabsorption to maintain neutral Pi balance.

In addition to hypocalcemia, also elevation of serum Pi stimulates PTH secretion both directly and indirectly. The indirect mechanism of Pi to PTH secretion through lowering serum Ca concentration has been known for decades (Reiss *et al.* 1970). The decrease in serum Ca by dietary Pi is attributed to the formation of calcium phosphate complexes in the blood (Sax 2001). The direct effect of Pi on parathyroid glands both *in vitro* (Almaden *et al.* 1998) and *in vivo* (Estepa *et al.* 1999) was discovered more recently.

2.3.4.2 Calcitriol

Production of calcitriol from its precursor 25-hydroxyvitamin D by 1-α-hydroxylation in the kidney is tightly regulated. It is induced by PTH and decreased serum Ca and Pi concentrations and suppressed by FGF-23 and increased serum Ca and Pi concentrations. In addition, two other phosphatonins, fibroblast growth factor 7 (FGF-7) and secreted frizzled-related protein-4 (sFRP-4), appear to inhibit synthesis of calcitriol (Bergwitz & Jüppner 2011). FGF-23 and calcitriol also increase conversion of 25-OH-vitamin D and calcitriol into inactive metabolites.

Calcitriol has both direct and indirect effects on Pi metabolism. Calcitriol affects serum Pi directly by increasing its intestinal absorption, but also indirectly by increasing its tubular reabsorption through suppressing PTH. The opposing effects of PTH and calcitriol on the kidney and the intestine, respectively, balance serum Pi concentration while preserving Ca ion homeostasis (Penido & Alon 2012).

2.3.4.3 Fibroblast growth factor 23

Traditionally, the PTH-vitamin D axis described above has provided the basis for the understanding of bone and mineral homeostasis. However, discovery of FGF-23 has led to finding a bone-kidney axis regulating principally Pi, vitamin D metabolism, and mineralization of bone (Martin *et al.* 2012). This hormonal cascade links bone to several other organ functions through a complex endocrine network integrated with the PTH-vitamin D axis. FGF-23 is mainly produced by osteoblasts and osteocytes (Yoshiko *et al.* 2007), even though it is also expressed by salivary glands, by the stomach, and at much lower concentrations by other tissues such as skeletal muscle, brain, mammary gland, liver, and heart (Martin *et al.* 2012).

Calcitriol is the most important systemic regulator of FGF-23; it seems to directly stimulate FGF-23 production in bone (Liu *et al.* 2006). In contrast, the effect of Pi on FGF-23 is not completely clear. In patients with renal failure, FGF-23 concentrations are elevated, and the degree of elevation correlates with the degree of hyperphosphatemia (Weber *et al.* 2003). However, in healthy humans, dietary Pi may not affect FGF-23 secretion (Larsson *et al.* 2003), at least not acutely (Nishida *et al.* 2006, Lamberg-Allardt *et al*., unpublished results). On the other hand, dietary Pi intake has been shown to regulate serum FGF-23 concentration in longer-term studies on healthy individuals (Ferrari *et al.* 2005, Antoniucci *et al.* 2006, Burnett *et al.* 2006). Pi load, rather than serum concentration, has been speculated to regulate FGF-23, as FGF-23 may vary in animals and patients without any changes in serum Pi (see Martin *et al.* 2012). Furthermore, Martin *et al.* (2012) suggest that Pi only has indirect effects on FGF-23 production, mediated through the effects of Pi on extracellular matrix mineralization.

Another systemic regulator of FGF-23 production is leptin, a hormone secreted by white adipose tissue to control energy intake and expenditure and bone homeostasis. Leptin stimulates FGF-23 production in bone (Tsuji *et al.* 2010). Similarly, oestrogens may be a potent stimulator for FGF-23 (Carrillo-Lopez *et al.* 2009). FGF-23 expression is downregulated, through yet unknown mechanisms, by phosphate regulating gene with homologies to endopeptidases on the X chromosome (PHEX) and dentin matrix protein 1 (DMP1), both of which are expressed by osteoblasts and osteocytes (Martin *et al.* 2012).

FGF-23 is secreted in the systemic circulation. In its target tissues, FGF-23 binds to and activates FGF receptors when its essential co-factor Klotho is present (Penido & Alon 2012). Klotho is a transmembrane protein highly expressed in the kidney and, to a lesser extent, in the parathyroid glands and brain. Klotho forms complexes with FGF receptors and increases their affinity for FGF-23. Hence, whether Klotho is present or absent dictates which tissues will respond to FGF-23. Abnormalities in either FGF-23 or Klotho production or function underlie many inherited and acquired disorders of phosphate homeostasis (Shaikh *et al.* 2008, Martin *et al.* 2012).

FGF-23 acts in the renal proximal tubule to reduce the expression of Npt2a and Npt2c, leading to decreased Pi reabsorption (Saito *et al.* 2003). FGF-23 also reduces the production of calcitriol and increases its catabolism to inactive metabolites by inhibiting the activity of 1-α hydroxylase and stimulating that of 24-hydroxylase (Shimada *et al.* 2004). Through the reduction in serum calcitriol, FGF-23 further reduces the intestinal absorption of Pi (Saito *et al*. 2003). FGF-23 may also inhibit PTH secretion (Ben-Dov *et al.* 2007). In summary, the main function of FGF-23 and the reason for its predominant expression in bone are to protect the cells from the toxic effects of excess Pi and calcitriol (Martin *et al.* 2012). More specifically, FGF-23 acts as a counter-regulatory hormone for calcitriol and coordinates renal Pi handling to match bone mineralization. Still, there is much to be discovered about FGF-23 regulation and function such as the interrelationships between bone remodeling, PTH, vitamin D, Klotho, and FGF-23. The current understanding of the regulation of P homeostasis is shown in Figure 3.

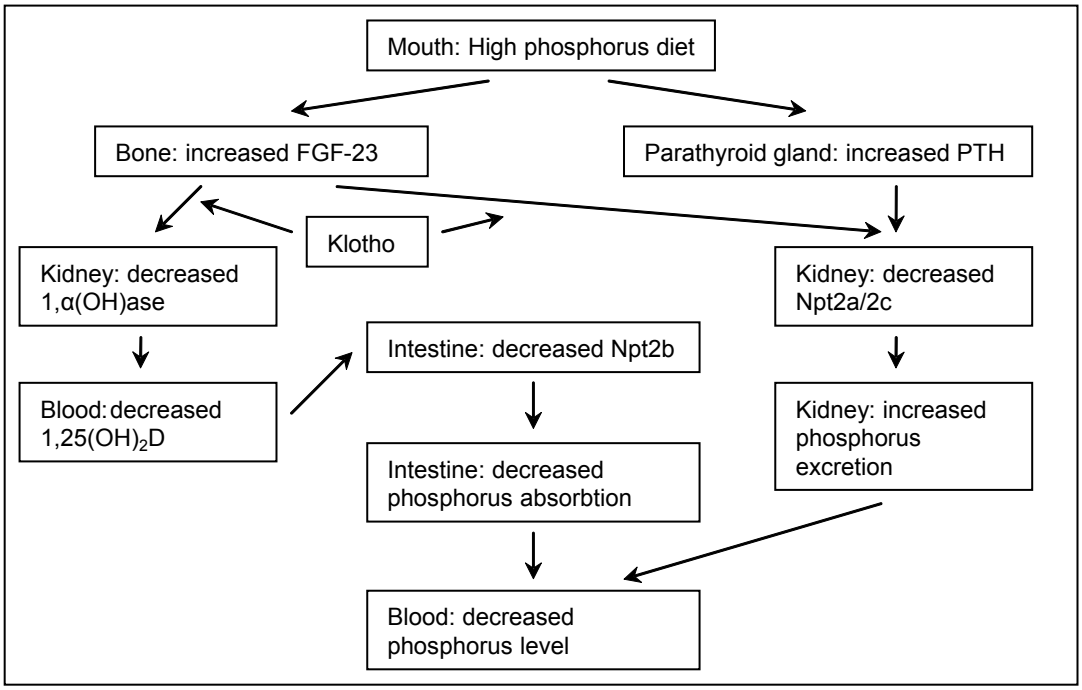

Figure 3 *Regulation of phosphorus homeostasis (adapted from Lien 2013).*

2.3.4.4 Other

To date, three phosphatonins in addition to FGF-23 have been identified: secreted frizzled related protein-4 (sFRP-4), matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor 7 (FGF-7) (Shaikh *et al.* 2008). Like FGF-23, sFRP-4 decreases renal Pi reabsorption by reducing Npt2a in renal proximal tubules and suppresses the

synthesis of calcitriol. MEPE increases the fractional excretion of Pi, inhibits bone formation *in vitro,* and inhibits intestinal Npt2b expression (see Section 2.3.2), but does not inhibit calcitriol formation. FGF-7 is able to inhibit Npt transport in animal kidney cells, but like MEPE does not influence the synthesis of calcitriol (Carpenter *et al.* 2005). Renal Pi handling is also affected by other systemic hormones such as insulin, growth and thyroid hormone, calcitonin, and glucocorticoids (see Section 2.3.2).

2.4 Bone metabolism

Bone is a specialized connective tissue, which together with cartilage makes up the skeletal system. Bone is constituted of bone cells (2-5% of volume) and the extracellular matrix (95-98% of volume), the latter consisting of mineral-covered protein matrix (Heaney 1999). The protein matrix is formed of collagen fibres (type I, 90% of total protein) and non-collagenous proteins. The mineral content of bone is mostly hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Osteocytes, i.e. bone cells, are found embedded deep within the bone in small lacunae. Osteocytes have numerous long cell processes that form a network of thin canaliculi permeating the entire bone matrix. Due to this architecture, all normal bone is in contact with living cells.

The calcified bone matrix is not metabolically inert. Most of the bone tissue turnover occurs at the bone surfaces, particularly at the endosteal surface where it interfaces with bone marrow. Two types of bone tissue exist: cortical bone, which is mainly found in the diaphysis of long bones, and trabecular bone, which exists more commonly in the metaphysis and epiphysis of long bones and vertebrae (Clarke 2008). Cortical bone is typically less metabolically active than trabecular bone.

Osteoblasts are the bone lining cells that lay down bone by synthesizing, depositing, and orienting the proteins of the matrix, then initiating changes for matrix mineralization (Heaney 1999). Osteoblasts secrete alkaline phosphatase to hydrolyse organic Pi compounds, which would otherwise inhibit mineralization. After stopping matrix synthesis, osteoblasts become osteocytes. Osteoclasts are cells that resorb bone by secreting hydrogen ions and proteolytic enzymes, such as cathepsin K, into the resorption compartment beneath them. After a cycle of resorption, osteoclasts undergo apoptosis, leaving their excavation to be refilled by osteoblasts. The process of removal of old bone, replacing it with newly synthesized proteinaceous matrix, and subsequent mineralization of the matrix to form new bone, is called remodelling. By remodelling, bone is renewed to maintain bone strength and mineral homeostasis. Modelling is a similar process to remodelling, except that during modelling the new bone is laid down at a location different from the site of resorption, and bone formation and resorption are not tightly coupled (Clarke 2008). By modelling, bones change their shape during growth.

2.4.1 Regulation of bone metabolism

Both systemic and local regulators affect bone cell function. Of systemic regulators of bone metabolism, the most important hormone is PTH, which stimulates bone formation when given intermittently and bone resorption when secreted continuously (Hock & Gera 1992). By stimulating absorption of Ca and P, calcitriol maintains adequate serum concentrations of these minerals, allowing passive mineralization of the unmineralized bone matrix. Serum calcitriol also promotes differentiation of osteoblasts and stimulates the expression of alkaline phosphatase and several cytokines (Clarke 2008). Calcitonin inhibits the function of osteoclasts only at pharmacologic doses (Hadjidakis & Androulakis 2006). Growth hormone is essential for skeletal growth, affecting both bone formation and resorption. Glucocorticoids have stimulatory as well as inhibitory effects on bone cells. However, glucocorticoid treatment is associated with increased bone resorption and risk of ostoporosis (Canalis *et al.* 2007). Thyroid hormones stimulate both bone resorption and formation, causing increased bone turnover in hyperthyroidism (Hadjidakis & Androulakis 2006). Oestrogens affect bone cells through several mechanisms, resulting in prevention of osteoclast formation and inducing their apoptosis, but also stimulating osteoblast proliferation and increasing their life span. Hence, inadequate oestrogen is associated with increased osteoclastic bone resorption. Androgens have similar effects on bone. In addition, they are essential in bone growth.

FGF-23 affects bone indirectly through its effects on calcitriol and PTH. Both under- and over-expression of FGF-23 have been shown to result in impairments in bone metabolism (Sitara *et al.* 2004, Liu *et al.* 2006b). Despite reported Klotho absence from mineralized tissues, there is debate regarding whether FGF-23 also affects bone directly (Martin *et al.* 2012). Results from animal models and cell studies suggest that FGF-23 inhibits mineralization in bone directly, independent of Pi homeostasis (Sitara *et al.* 2008, Shalhoub *et al.* 2011).

Of local regulators of bone cells, osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK) system is vital in the control of osteoclastogenesis and bone remodelling in general (Hadjidakis & Androulakis 2006). Preosteoblastic/stromal cells express RANKL, which interacts with RANK. The RANKL/RANK interaction results in osteoclastogenesis and promotion of osteoclast survival. OPG, a glycoprotein mainly produced by cells of the osteoblast lineage, inhibits the entire system by blocking the effects of RANKL. OPG and RANKL may act as the mediators for the stimulatory or inhibitory effects of a variety of systemic hormones, growth factors, and cytokines on osteoclastogenesis. For example, PTH increases the production of RANKL and decreases that of OPG.

Other central local factors affecting bone metabolism are macrophage colony-stimulating factor (M-CSF), which appears to be necessary for osteoclast development, and several cytokines, such as tumour necrosis factor α (TNF-α) and interleukin 10 (IL-10), which

stimulate M-CSF production and increase RANKL expression (Hadjidakis & Androulakis 2006). Interleukin 6 (IL-6), another cytokine secreted by osteoblasts, osteoclasts, and stromal cells, stimulates osteoclastic bone resorption and osteoblast generation in conditions of high bone turnover.

2.4.1 Markers of bone metabolism

Currently available markers of bone turnover include both enzymes and non-enzymatic peptides derived from cellular and non-cellular compartments of bone (Seibel 2005). They are usually classified as markers of bone formation or bone resorption. This distinction is not, however, absolute; some markers, at least partly, reflect both bone formation and resorption (e.g. certain osteocalcin fragments).

Markers of bone formation are products of active osteoblasts considered to reflect different aspects of osteoblast function and bone formation (Seibel 2005). All bone formation markers are measured in serum or plasma. Serum total alkaline phosphatase (AP) is the most widely used marker of bone metabolism due to its simple and inexpensive detection methods. The exact function of the enzyme is unknown. As various tissues produce AP, techniques for analysing the bone-specific isoform of AP (BALP) have been developed, even though a good correlation between BALP and total AP has been found in healthy adults (Woitge *et al.* 1996). Another commonly used bone formation marker is osteocalcin (OC), which is a hydroxyapatite-binding protein exclusively synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes (Seibel 2005). OC is considered a specific marker of osteoblast function, and newer assays measuring its mid-molecule eliminate in part the problem of pre-analytical instability. Osteoblasts synthesize type I collagen from its precursor molecules characterized by the amino-terminal propeptide (PINP) and the carboxy-terminal propeptide (PICP). These peptides are also used as markers of bone formation.

Many bone resorption markers are degradation products of bone collagen (Seibel 2005). However, non-collagenous proteins, such as bone sialoprotein, and osteoclast-derived enzymes, such as cathepsin K and L, have recently been investigated as markers of bone metabolism. Pyridinolines (Pyr) and deoxypyridinolines (DPyr) are collagen crosslink components that are proteolytically broken down during bone resorption. Pyr and DPyr are highly specific to skeletal tissues, and they can be measured in serum or urine samples. Carboxy- and amino-terminal telopeptides of type I collagen (CTx and NTx, respectively) measured either in serum or urine are also used as bone resorption markers. Tartrate-resistant acid phosphatase (TRACP) is an enzyme with two known subforms, 5a and 5b, the latter of which is characteristic of osteoclasts. TRACP can be measured in serum or plasma.

The problem regarding the use of bone markers is the large within-subject variability (Seibel 2005). To deal with this variability, diurnal and seasonal variation, the age and sex of the subject, and menstrual cycle in premenopausal women should be taken into account when interpreting the results. In addition, standardized sampling and sample handling are required to obtain reliable results. It should also be noted that abnormal kidney function affects concentrations of bone markers.

2.5 Disorders involving phosphorus

2.5.1 Chronic kidney disease

Chronic kidney disease (CKD) is the progressive loss of renal function due to an irreversible and progressive glomerular, tubular, or interstitial disease (Edwards 2002). CKD is classified into stages 1-5 based on the presence or absence of biomarkers in blood or urine and the glomerular filtration rate (GFR) (Table 4, National Kidney Foundation 2002). GFR is usually estimated from serum creatinine by using either the Cockroft-Gault or the MDRD (Modification of Diet in Renal Disease) formula. Treatment in the early stages may prevent or delay the progression of CKD, but early detection is often difficult due to the asymptomatic nature of the disease. Stage 5, defined as end-stage renal disease (ESRD), often requires renal replacement therapy, i.e. dialysis or transplantation.

Based on epidemiological studies, the median prevalence of CKD is ca. 7% in persons aged 30 years or older, varying depending on ethnic background (Zhang & Rothenbacher 2008). Independently of age, hypertension and obesity are associated with an increased prevalence of CKD (Otero *et al.* 2010). Other risk factors of CKD include diabetes, dyslipidemia, and smoking (Foley *et al.* 2005, Kronborg *et al.* 2008, Otero *et al.* 2010). CKD is a risk factor for all-cause mortality, cardiovascular disease, and other comorbidities, and it substantially lowers the self-reported quality of life (National Kidney Foundation 2002). Furthermore, treatment of CKD and its complications is a tremendous economic burden globally (Jha *et al.* 2012).

Table 4. *Stages of chronic kidney disease.*

Stage	Description	GFR (ml/min/1.73 m^2)
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure	<15 (or dialysis)

Patients with CKD progressively lose the ability to excrete P (Shroff 2012). Decreased P excretion is initially compensated by increased PTH and FGF-23 secretion. As renal function declines, increasingly higher levels of PTH are needed to maintain Pi homeostasis. In the advanced stages of renal disease, the elevated levels of PTH are unable to maintain normal S-Pi concentrations and hyperphosphatemia becomes evident. Hyperphosphatemia is associated with dysregulation in Ca, PTH, and vitamin D homeostasis. These changes can result in bone demineralization and ectopic soft tissue calcification, leading to the complex of CKD – mineral and bone disorder (CKD-MBD) (Moe *et al.* 2006). The biochemical changes in CKD-MBD are summarized in Figure 3.

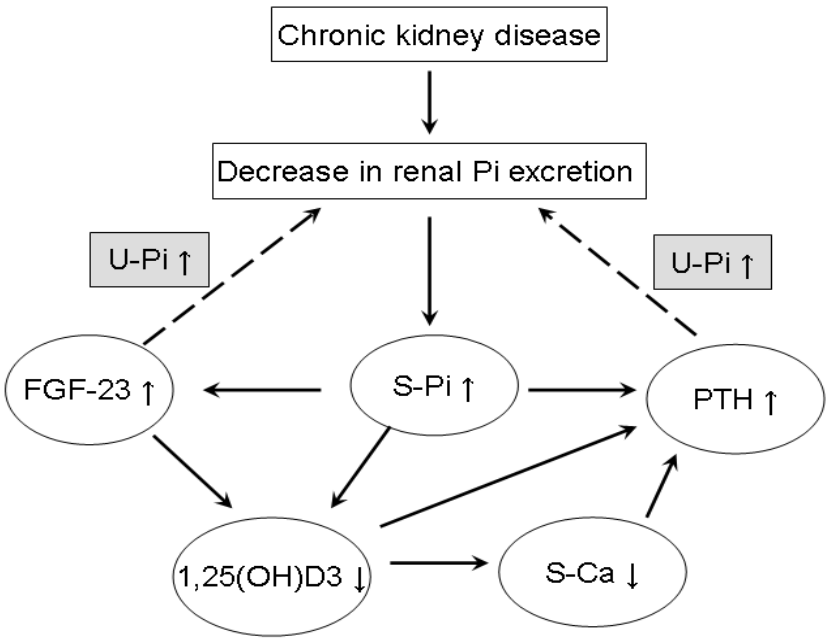

Figure 4 *Biochemical changes in CKD-MBD (adapted from Keronen et al. 2012).*

Vascular calcification in CKD patients occurs in both the intima and media layers of the vessel wall. Intimal deposition is associated with atherosclerotic plaques, while medial deposition is associated with vascular stiffening and arteriosclerosis. Vascular calcification largely explains the early cardiovascular morbidity and mortality of CKD patients (Blacher *et al.* 2001), and it occurs decades earlier than in the general population (Goodman *et al.* 2000). In a longitudinal cohort study, higher serum P levels were associated with a greater risk for end-stage renal disease and mortality in subject with normal kidney function (Sim *et al.* 2013), indicating that dietary P could play a role also in the aetiology of CKD. Early treatment of Pi retention or hyperphosphatemia may prevent or slow the development of CKD-MBD (Martin & González 2011). Pi retention is treated with dietary P restriction, limiting intestinal P absorption by using oral P binders (usually Ca acetate or Ca carbonate) and, at stage 5, dialysis. However, the efficacy of current P binders is limited, and dialysis is not sufficient to maintain a normal serum Pi concentration in a patient eating an average diet (Winger *et al.* 2011). Therefore, depending on the protein need of the patient, the recommended intake of P for a CKD patient is <800-1000 mg/d (National Kidney Foundation 2003, Finnish Kidney and Liver Association 2009).

Dietary P restriction is implemented by preferring products with the lowest possible P contents among both animal and plant protein sources and by avoiding P additive-containing foods (Finnish Kidney and Liver Association 2009, Kalantar-Zadeh *et al.* 2010). When dietary P is restricted, also protein intake is reduced, as protein-rich foods are major P sources. At CKD stages 2-4, protein restriction (0.6-0.8 g/kg/d) is beneficial, as a low dietary protein intake slows the progression of the disease (Fouque & Laville 2009). However, the protein need for ESRD patients in dialysis is higher (ca. 1.2 g/kg/d) because dialysis removes not only P, but also protein and amino acids from the blood (National Kidney Foundation 2003, Finnish Kidney and Liver Association 2009). To facilitate implementing appropriate protein and P intakes in CKD, a ratio of P (mg) to protein (g) may be a more suitable measure than the P content of a food alone (Kalantar-Zadeh *et al.* 2010). The recommended P-to-protein ratio for CKD patients is ≤ 15 (Finnish Kidney and Liver Association 2009). Still, for better outcomes of the nutritional care of CKD patients, a need for better knowledge of P bioavailability from different foods, more accurate reporting of P contents of foods by manufacturers, and development of lower P foods by the food industry exists (Kalantar-Zadeh *et al.* 2010, Winger *et al.* 2011).

2.5.2 Osteoporosis and bone health

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, which result in increased bone fragility and fractures (WHO 2003). BMD can be measured by dual x-ray absorptiometry (DXA), and osteoporosis is defined as BMD 2.5 SD below the average of a young adult. However, early osteoporosis is usually asymptomatic and remains undiagnosed until fractures occur. Osteoporotic fractures most commonly occur in the spine, hip, or wrist,

resulting in loss of mobility and autonomy and poorer quality of life. Furthermore, hip and spine fractures increase 12-month mortality risk due to hospitalization and chronic immobilization (Center *et al.* 1999).

Concern about high P intake and its role in the development of osteoporosis originates from animal studies. Development of secondary hyperparathyroidism with bone loss has been consistently documented in several species of animals fed a P-rich diet (Sie *et al.* 1974, Cook *et al.* 1983, for review, see Calvo & Park 1996). More recently, a high P intake has been observed to impair growth and result in poorer bone quality in rats (Huttunen *et al.* 2007). A rise in serum PTH concentration after oral Pi load has also been found in humans (Reiss *et al.* 1970, Brixen *et al.* 1992, Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006), which, in the long run, increases bone resorption. In a short-term controlled study, the effect of Pi on S-PTH has been shown to be dose-dependent (Kemi *et al.* 2006). However, results of several other experimental human studies on dietary P are confounded by not controlling for Ca intake (Silverberg *et al.* 1986, Silverberg *et al.* 1989, Brixen *et al.* 1992) or by simultaneous changes in both Ca and P intakes (Calvo *et al.* 1988, Calvo *et al.* 1990). Still, cross-sectional studies support the findings of P on PTH (Kemi *et al.* 2009, Kemi *et al.* 2010). Dietary P seems to affect PTH secretion more strongly in women than in men (Calvo *et al.* 1988, Whybro *et al.* 1988), and in older subjects than in younger subjects (Silverberg *et al.* 1989, Renier *et al.* 1992). In addition to increased PTH secretion, dietary P load decreases serum calcitriol concentration, as observed in healthy men (Portale *et al.* 1986) and in young (Kemi *et al.* 2006) and postmenopausal women (Silverberg *et al.* 1989).

Divergent results on effects of P on markers of bone turnover have been reported. In the short-term study of Kärkkäinen & Lamberg-Allardt (1996), a single dose of 1500 mg of P decreased markers of bone formation (BALP and PICP), but did not affect markers of bone resorption. In the similar study of Kemi *et al.* (2006), both 750 mg and 1500 mg doses of P decreased S-BALP, and the 1500 mg P dose increased urinary NTx, indicating increased bone resorption and decreased bone formation. In most other studies, changes in markers of bone metabolism were not found (Calvo *et al.* 1990, Brixen *et al.* 1992, Whybro *et al.* 1998, Grimm *et al.* 2001). In addition to differing Ca and P intakes and study designs, the power of the studies may have been too low to detect differences in the bone markers. The current more sensitive markers of bone metabolism could more easily disclose changes with smaller numbers of subjects.

Some studies have examined the effects of dietary P on BMD. In cross-sectional studies of postmenopausal (Tylavsky & Anderson 1988) and young women (Metz *et al.* 1993), radial BMD was negatively associated with P intake. Moreover, a high dietary Ca:P ratio (1.2-1.4) was related to higher total body BMC and BMD in multiple regression analyses in a cross-sectional study of young women (Teegarden *et al.* 1998). An association between low dietary Ca:P ratio and low BMD has also been observed in perimenopausal women (Brot *et al.* 1999). As cola drinks contain phosphoric acid, and hence, are a Ca-free P

source, their relationship to bone health has been investigated in some studies. In the Framingham Osteoporosis Study, a dose-response relationship was observed between the amount of cola (also decaffeinated and diet cola) consumed and BMD in 29- to 83-year-old women, but not in men (Tucker *et al.* 2006). The inverse association was not seen with use of non-cola soft drinks, which suggests phosphoric acid as the factor behind the results concerning cola beverages. Similar results were reported in adolescent girls, in whom higher intakes of carbonated soft drinks were associated with lower heel BMD (McGartland *et al.* 2003). Such an association was not found in boys. Earlier, however, no association was observed between cola drink consumption and BMD in women aged 44-98 years (Kim *et al.* 1997).

Data on P intake and fracture risk are scarce. In a follow-up study among middle-aged and elderly men, the lowest P intake quintile was associated with an increased fracture risk compared with subjects in the second quintile (Elmståhl *et al.* 1998). Greater risk of fractures has been found in former female athletes (Wyshak *et al.* 1989), and in adolescent girls (Wyshak & Frisch 1994, Wyshak 2000) who consumed carbonated beverages compared with those who did not. However, the study of Wyshak (2000) has been criticized for methodological problems (Williams 2001). Furthermore, according to Fitzpatrick & Heaney (2003), the association between soft drinks and BMD or fractures is more likely due to displacement of milk consumption than to a direct effect of soft drink components. The P contents of cola and other soft drinks are smaller than those of several other beverages, including milk, which supports this opinion.

To conclude, the data especially from human studies on P intake and bone health are inconsistent and insufficient to draw definite conclusions. More population-based and controlled long-term intervention studies on skeletal variables and fractures are needed to address the effect of high P intake on bone.

2.5.3 Cardiovascular disease

The role of P in the vascular component of CKD is well established. In recent studies, higher serum Pi levels even within the normal range have been associated with increased risks of cardiovascular events in healthy populations with intact kidney function (Foley *et al.* 2008, Dhingra *et al.* 2008, Foley *et al.* 2009, Larsson *et al.* 2010). Furthermore, higher serum Pi levels have been shown to be associated with risk of death and cardiovascular events in adults with prior myocardial infarction (Tonelli *et al.* 2005). Similar results in cardiovascular mortality have been found in type 2 diabetics (Chonchol *et al.* 2009). On the other hand, in a case-control study, serum Pi levels were not associated with the development of incident coronary heart disease in men without CKD (Taylor *et al.* 2011).

Several mechanisms may explain the association between S-Pi increases and cardiovascular events. Firstly, abnormal Pi handling may contribute to the initiation and/or

progression of vascular calcification by multiple mechanisms. Phosphate may induce phenotypic changes in vascular smooth muscle cells (VSMCs), causing them to transform from a contractile phenotype to an osteochondrogenic phenotype (Kendrick & Chonchol 2011). Furthermore, Pi has been hypothesized to induce apoptosis of VSMCs, which could accelerate the calcification process. Elevated Pi concentrations may also decrease the differentiation of monocytes/macrophages of the vessel wall into osteoclast-like cells. It has been speculated that this reduction in osteoclast activity may be an additional way that elevated Pi levels result in vascular calcification, as osteoclast-like cells prevent mineral deposition in the vasculature. High serum FGF-23 concentrations are linked to death and cardiovascular events by unknown mechanisms. Hence, Pi-mediated elevation of FGF-23 has been suggested to be another factor affecting vascular calcification. In addition, Pi may be related to vascular calcification through Klotho, which may inhibit calcification directly by affecting VSMCs, and indirectly by controlling S-Pi through its interaction with FGF-23. In healthy human subjects, an oral Pi supplement that increased S-Pi to the upper limit of the normal range was associated with a transient reduction in endothelium-dependent brachial artery dilation, suggesting that acute postprandial hyperphosphatemia mediates endothelial dysfunction (Shuto *et al.* 2009). A recent rat study provided further support for the role of Pi in endothelial function, as dietary P restriction ameliorated endothelial dysfunction in kidney diseased rats (Van *et al.* 2012).

The associations discussed above are between cardiovascular events and S-Pi rather than dietary intake, and dietary P sources may complicate these findings (Ellam & Chico 2012). Furthermore, there is a need to understand the contribution of each component of the Pi regulatory axis (PTH, FGF-23, calcitriol) to any causative mechanisms of atherogenesis in order to optimize the whole axis. The question remains as to whether reducing S-Pi prevents or reverses cardiovascular risk factors.

3 Aims of the study

The objective of this thesis was to study total and *in vitro* digestible P content (TP and DP, respectively) of foods and the effects of different P sources on mineral metabolism of healthy subjects.

Specific research questions were as follows:

Study I: Are there differences in TP and DP content of selected commonly used plant foods and beverages?

Study II: Are there differences in TP and DP content of selected commonly used meat and milk products?

Study III: Do different P sources affect markers of P, Ca, and bone metabolism differently in healthy young females?

Study IV: Do mono- and polyphosphates affect markers of P and Ca metabolism differently in healthy young females?

4 Subjects and methods

4.1 Food studies (Studies I and II)

4.1.1 Foods chosen for phosphorus analyses

Foods considered relevant as P sources were chosen for analysis. Meat, milk, grains, and legumes are important P and protein sources; thus, products from these food groups were analysed. In addition, foods containing P additives, i.e. cola beverages, processed cheeses, and meats, were analysed. To clarify P contents of similar products, several labels among processed meats, cola beverages, cheeses, and breads were evaluated.

To improve representativeness of the analyses, we focused on the nationally most popular products in each food category. Market shares of the products were found on the basis of a substudy of the national dietary survey FINDIET (Reinivuo *et al.* 2010). The main aim of the substudy was to investigate the market shares of food products. For this purpose, a subsample (50%) of the participants (n=1025) of the FINDIET Study filled in a 5-day product purchase diary to investigate the purchase and use of voluntarily fortified foods. A total of 930 subjects (91%) returned the diary, 918 (90%) of which were of acceptable quality. Each food item was assigned a food identifier from the Finnish Food Composition Database (Fineli), enabling data classification. Market shares of each product were calculated by dividing the total amount (kg) of purchased product by the total amount of purchased food in the same food class.

Two to seven of the most popular trademarks (highest market shares) were pooled for each sample item. The leading trademark in each sample category was analysed as such. The chosen sample items were purchased from grocery stores in the Helsinki area. The number of purchased trademarks was twice the proportion of that item in the pool (40% in the pool corresponding to 8 subsamples). The minimum was two subsamples for each pooled category per trademark. The samples were homogenized in a blender, packed into polyethylene bags, and stored at -20ºC until analysis.

We hypothesized that the use of P-containing additives increases both total and *in vitro* digestible P content (TP and DP, respectively) of the food compared with products prepared without P additives. Furthermore, we hypothesized that the proportion of DP to TP is lower in plant-based P additive-free products than in products of animal origin.

4.1.2 Analysis of total phosphorus in foods

The food sample (0.5 g) was digested in a microwave oven with 10 ml of concentrated nitric acid. The sample was transferred to a 50-ml volumetric flask with purified water, and the content of P was analysed with ICP-OES. The quantitation was performed by using an external calibration curve with five different concentrations. P was analysed radially with wavelengths of 177.4, 178.2, 178.7, and 213.6 nm. The results were calculated as a mean. All treatments of the samples were with five replicates. The fat was removed from samples if the fat content was more than 6-8%. Petroleum ether (200 ml, bp: 60ºC to 71ºC) was added and the suspension was agitated with a magnetic stirrer at room temperature for 15 min. The solvent was filtered and the sample was air-dried at room temperature overnight.

The accuracy and precision of the total P (TP) content of the samples were tested using NBS 1567a wheat flour reference material and one in-house reference material (flour I) in every P determination batch. TP contents of analysed foods were calculated per 100 g of the product. TP-to-protein ratios (mg/g) were calculated. Protein and sodium contents of foods were taken from either food labels or the food composition database.

4.1.3 Analysis of in vitro *digestible phosphorus in foods*

The samples were treated according to the method described by Asp *et al.* (1983) for dietary fibre, with some modifications for the determination of DP (Ekholm *et al.* 2000). In this method, starch and proteins of the sample were digested enzymatically, in principle, in the same way as in the alimentary canal. The samples were then dialysed and DP was analysed from the dialysate by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6000 Series ICP Spectrometer, Waltham, MA, USA). The insoluble mineral bound by food components remained in the dialysis residue.

The homogenized and ground sample was weighed into a 100-ml flask. In all, 50 ml of purified water (MilliQ) was added, and the starch was gelatinized by incubating the sample in a boiling water bath for 15 min. Then 0.5 ml of the thermal stabile amylase enzyme (Termamyl 300 L, Novo Nordisk A/S Bagsvaerd, Denmark) was added, and the mixture was further incubated in the boiling water bath for 1 h 45 min to hydrolyse the starch. The amount of amylase enzyme was kept as low as possible to minimize contamination by Ca and P originating from the enzyme. The pH of the reaction mixture was then adjusted to 1.5 with 1 M HCl. After addition of 0.5 g of Pepsin (0.7 FIP-U/mg, EC 3.4.23.1, Merck, Darmstadt, Germany), the mixture was incubated at 40ºC for 60 min. The pH was increased to 6.8 with 1 M NaOH, and 0.5 g of pancreatin enzyme (Sigma 8 X 3 U.S.P, St. Louis, MO, USA) was added and the sample mixture was kept for an additional 60 min at 40ºC. The volume of the samples was adjusted to 100 ml with

purified water, and 50 ml of the mixture was dialysed against purified water overnight at room temperature. The concentrations of P in the dialysate were determined by ICP-OES as described in Section 4.1.2.

The uncertainty of the method in determining the digestibility of P was 7.4%, and it was determined using an in-house reference sample (wheat flour) in every analysed batch and calculated according to Eurachem (Eurachem/Citac 2000). DP contents of analysed foods were calculated per 100 g of the product. The proportions of DP to TP were determined. DP-to-protein ratios (mg/g) were calculated.

4.2 Human studies (Studies III and IV)

4.2.1 Subjects

The subjects participating in the studies were healthy 19- to 31-year-old female volunteers recruited from the University of Helsinki Viikki campus area. The participants had no medications or illnesses known to affect calcium or bone metabolism other than hormonal contraceptives. Before the beginning of the study, the subjects kept a 4-day food record, for which they received instruction both in writing and verbally. The habitual dietary intake of the subjects was calculated with computer-based programs Diet 32 (Aivo 1.2.2.2, AIVO Finland Oy, Turku, Finland) (Study III) and Flamingo (version 0.5.6, Helsinki, Finland) (Study IV). Background information, such as weight and height of the volunteers, was collected with self-reported questionnaires.

4.2.1.1 Ethical considerations

The studies were conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Ethics Committee of Public Health and Epidemiology in the Hospital Districts of Helsinki and Uusimaa (Study III) and the Ethics Committee of the Faculty of Agriculture and Forestry (Study IV). Written informed consent was obtained from all subjects prior to their inclusion in the study. Subjects could withdraw their participation without a reason at any time. After the study, they were provided with the results of their personal nutrient intakes based on the food record.

4.2.2 Study designs and hypothesis

The studies were controlled short-term (24-h) studies in which subjects served as their own controls. The subjects attended the 24-h sessions at 7-day intervals. To exclude the effect

of alcohol on PTH secretion, the subjects were advised not to drink alcohol 24 h before the beginning of the study sessions. In addition, the subjects were asked to maintain consumption of milk products at their usual level before the study sessions to prevent exceptional Ca intakes that could have affected PTH secretion. During the study sessions all meals were provided. Except for supper, all meals were eaten at the research unit. The meals were prepared from normal foods purchased from local grocery stores, and prepared and measured by the same person on each study day. No additional meals or snacks were allowed, but water was provided *ad libitum*. The food composition database (Fineli®) of the National Institute for Health and Welfare and manufacturers' information were used in designing the study diets. In addition, the P concentrations of the whole-grains, meats, and cheeses used in Study III were analysed at the laboratory of the Finnish Meat Research Institute.

4.2.2.1 Study III

In Study III, sixteen healthy female volunteers participated in five separate 24-h sessions. Before coming to the research unit, subjects fasted overnight. At the control session, both Ca and P intakes were low (approximately 250 and 500 mg/day, respectively). During the other four sessions P intake was approximately 1500 mg/day, 1000 mg of which was obtained from meat, cheese, whole-grains, or a phosphate supplement, respectively. The order of the study sessions was randomized. The foods served during P sessions and the control session were identical, with the exception of differing P sources. P intake was divided evenly between these meals at all sessions. The schedule of a study session is summarized in Figure 5.

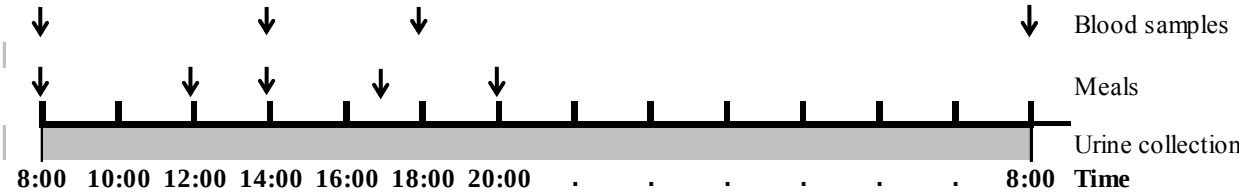

Figure 5 *Schedule of study days in Study III.*

At the meat session, the main P sources were ham and beef steak, both prepared without P additives. At the cheese session, the subjects were served fermented cheese (Edam type, 17% fat). At the whole-grain session, the main P sources were oatmeal porridge and non-fermented rye bread. The P supplement was prepared from a mixture of disodium and trisodium phosphates (E450 and E451; Six Oy, Helsinki, Finland) and served in a refreshment drink. To increase protein intake during the control, whole-grain, and supplement sessions, cooked egg white was served to subjects because egg white has a high protein but very low phosphorus content. Energy intakes were balanced by using different amounts of oil, margarine, sugared and diluted berry juice, berry soups, and

canned fruit. The diets during each session contained the same amounts of sodium (3.8 g/day). The amount of daily protein consumed was similar (82-95 g/day) during all sessions other than the meat session, when protein intake was higher (125 g/day) because of the high protein content of meat. Likewise, fibre intake was higher (59 g/day) during the whole-grain session than during the other sessions (19-21 g/day). The Ca intake during the cheese session was high (2044 mg/day). The intakes of selected nutrients during the study sessions are presented in Table 5. We hypothesized that depending on differences in P absorbability, the P sources have different effects on mineral metabolism.

Table 5. *Intakes of selected nutrients during the study sessions (Study III).*

Variable	Control	Meat	Whole grains	Cheese	Supplement
Energy (MJ)	8.5	8.4	8.4	8.5	8.8
Protein (g)	82	125	82	88	95
Carbohydrate (g)	266	237	278	260	289
Fat (g)	68	60	60	68	59
Ca (mg)	235	274	352	2044	246
P (mg)	484	1435	1509	1624	1458
Na (mg)	3826	3855	3850	3833	3824
Fibre (g)	20	19	59	21	19

4.2.2.2 Study IV

Fourteen healthy female volunteers participated in three separate 24-h sessions. During each session the subjects ingested either a monophosphate (MP), polyphosphate (PP) or placebo supplement during three meals. The MP supplement contained sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$, E339, Merck Eurolab, Germany) and the PP supplement sodium tripolyphosphate ($Na_5P_3O_{10}$, E452, Six Oy, Finland). The Pi and placebo drinks were divided into three doses, and served at 08:00, 12:00, and 16:00 hours with meals. The schedule of a study session is shown in Figure 6.

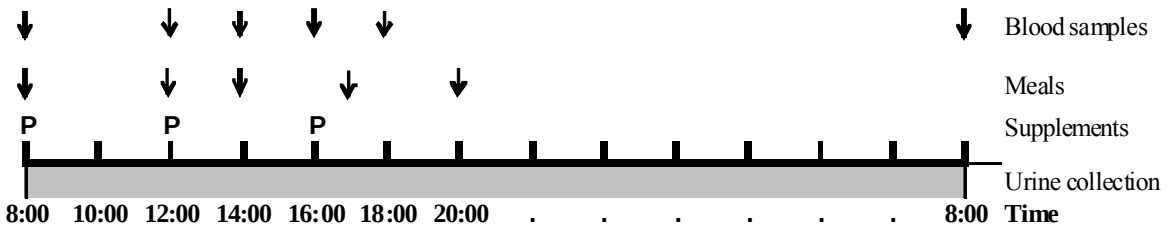

Figure 6 *Schedule of study days in Study IV.*

The Pi supplements contained 1500 mg/d P, each dose containing 500 mg of P. The meals served during each study session were identical, providing 340 mg of Ca and 500 mg of P. Since the Pi supplements also contained Na, the Na intakes were higher during the MP and PP sessions than during the placebo session. The total intake of Na (diet + supplements) during the placebo session was 1.4 g, during the MP session 2.5 g, and during the PP session 3.2 g. The intakes of selected nutrients during the study sessions are presented in Table 6. We hypothesized that the PP supplement would decrease Ca absorption, and consequently, increase the S-PTH concentration more than would the MP supplement.

Table 6. *Intakes of selected nutrients from the diet during the study sessions (Study IV).*

Variable	Intake
Energy (MJ)	8.1
Calcium (mg)	340
Phosphorus (mg)	500
Sodium (mg)	1370*
Fibre (g)	20

* During the phosphate supplement sessions sodium intake was higher since the phosphate supplements contained sodium.

4.2.2.3 Sampling

The timing of the blood samples is shown in Figure 5 (Study III) and Figure 6 (Study IV). The blood samples were taken before meals at all other times except at 18:00 hours, when, for practical reasons, it was taken immediately after the meal. Venoject gel tubes were used to obtain clear sera. Blood samples were handled anaerobically and centrifuged (3000 rpm for 15 min) within an hour of sampling. The 24-h urine collections were obtained on the study day from 08:00 to 08:00 hours the following morning. Both serum samples and portions of urine were stored at -20ºC until analysed.

4.2.3 Laboratory analyses

The serum ionized calcium (S-iCa) concentration was analysed from anaerobically handled serum samples with an ion selective analyser (Microlyte 6; Thermo Electron Oy, Vantaa, Finland) within 90 min of sample collection. In the other measurements, all samples from the same person were analysed in the same assay in randomized order. In Study III, intact S-PTH concentration was measured by an immunoenzymometric assay using Octeia® Intact PTH Kits (Immunodiagnostic Systems, Boldon, UK). In Study IV, the serum intact PTH concentration was measured by radioimmunoanalysis using Allegro intact PTH Assay Kits (Nichols Institute, San Juan Capistrano, CA, USA). Serum bone-

specific alkaline phosphatase (BALP) activity was determined only in Study III by an enzyme immunoassay (Metra™ BAP EIA Kit; Quidel, San Diego, CA, USA). Similarly, excretion of urinary N-terminal telopeptide of type I collagen (NTx) was analysed only in Study III by an enzyme-linked immunosorbent assay using Osteomark® NTx Test Kits (Ostex International, Seattle, WA, USA). Serum Pi and creatinine concentrations and excretions of urinary Ca, Pi, and creatinine were measured with an autoanalyser (Konelab 20, Thermo Electron, Vantaa, Finland).

All analyses were conducted at the laboratory of the Department of Food and Environmental Sciences, Division of Nutrition, University of Helsinki. Laboratory methods and intra- and inter CV% of methods are presented in Table 7.

Table 7. *Laboratory measurements and intra- and inter CV% of methods in Studies III and IV.*

Variable	Method	CV% intra		CV% inter	
Serum measurements		Study III	Study IV	Study III	Study IV
Serum ionized calcium (S-iCa)	Ion selective analyser	1.7	1.6	-	-
Serum intact parathyroid hormone (S-PTH)	IEMA (Study III) IRMA (Study IV)	1.0	2.9	4.2	5.1
Serum bone-specific alkaline phosphatase (S-BALP)	ELISA	5.4	-	7.4	-
Serum phosphate (S-Pi)	Spectrophotometric	1.1	1.4	1.4	3.3
Serum creatinine (S-Crea)	Spectrophotometric	2.4	-	1.1	-
Urinary measurements					
Urinary calcium (U-Ca)	Spectrophotometric	1.1	2.1	1.4	4.3
Urinary phosphate (U-Pi)	Spectrophotometric	1.7	2.8	3.5	3.5
Urinary creatinine (U-Crea)	Spectrophotometric	1.7	3.0	3.6	3.5
Urinary N-terminal telopeptide of type I collagen (U-NTx)	ELISA	4.6	-	6.4	-

ELISA= enzyme-linked immunoassay; IEMA=immunoenzymometric assay; IRMA=immunoradiometric assay; - not analysed

4.3.3 Statistical methods

The data are expressed as mean ± SEM (standard error of the mean). For serum variables, the area under the curve (AUC) for the difference from the morning fasting value was calculated. To exclude the effect of the first fasting sample, the deltas of the 0-h sample to

the 24-h sample were used in the analyses. In Study III, the morning fasting values of serum phosphate, iCa, PTH, and BALP from the second-day (24 h from the first blood sample) fasting samples were compared in addition to AUC values. Non-normal distributions were normalized by logarithmic transformations.

Analysis of variance (ANOVA) with repeated measures was used to compare the study sessions. If the sphericity assumption was violated, a Huynh–Feldt adjustment was applied. Contrast analysis was used for pairwise comparisons between study sessions. In Study III, analyses were conducted with the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL, USA), and in Study IV with PASW Statistics 17.0.2 (SPSS Inc., Chicago, IL, USA). Both versions of SPSS software were used in a Windows environment for all statistical analyses. We regarded $p<0.10$ as a trend and $p>0.05$ as a statistically significant difference.

5 Results

5.1 Total and *in vitro* digestible phosphorus content of foods (Studies I and II)

5.1.1 Meat and meat products

In sausages and cold cuts, the TP contents varied between 175 and 279 mg/100 g. The highest values were found in boiled ham containing phosphate additives. The TP content of a salami-type sausage was similar to that of a low-fat processed sausage, but the DP content of the former was 24% lower than that of the latter. The sodium content of the salami-type sausage (1.7 g/100 g) was the highest among foods analysed in this study. The P contents in frankfurters were slightly lower than those in other sausages or cold cuts. The TP contents varied between 199 and 232 mg/100 g in raw meats and rainbow trout. More variation seemed to exist in the DP contents of the meats (147 to 207 mg/100 g). The results of meat and meat products are shown in Table 8.

Table 8. *Total and in vitro digestible P, protein, P-to-protein ratios, sodium contents, and P additives of meats and meat products.*

Product	TP /100g	DP/100g	Protein/ 100g	TP-to-Protein Ratio (mg/g)	DP-to-Protein Ratio (mg/g)	Na (g/100g)	P additives[c]
Processed sausage, 18% fat	210	224	9[b]	23.3	24.9	0.9[b]	E450, E451
Processed sausage, light, 10% fat	241	242	10[b]	24.1	24.2	0.8[b]	E450
Frankfurter pool, 20% fat	175	144	9.2[b]	19.0	15.7	0.8[b]	E450, E451
Frankfurter pool, light, 13% fat	186	130	10[b]	18.6	13.0	0.7[b]	E450
Sausage, dry, salami type	244	171	21.5[b]	11.4	7.9	1.7[b]	E450
Sausage cold cuts	184	164	10.7[b]	17.2	15.4	0.8[b]	E450, E451
Boiled ham	279	255	17.9b	15.6	14.3	0.75[b]	E450, E451, E452
Raw pork steak	212	161	21[a]	10.1	7.7	0.06[a]	-
Raw chicken fillet	229	191	23[a]	10.0	8.3	0.08[a]	-
Raw beef	199	147	22[a]	9.1	6.7	0.04[a]	-
Raw rainbow trout fillet	232	207	16.8[a]	13.8	12.3	0.05[a]	-

[a]Protein and sodium contents based on Fineli®.

[b]Protein and sodium contents based on food labels.

[c]P additives listed in Table 1 in Appendix II.

TP = total phosphorus; DP = *in vitro* digestible phosphorus

5.1.2 Dairy products

The highest TP and DP contents were observed in cheeses. The TP contents of processed cheeses varied between 574 and 892 mg/100g, 89-100% of which was DP. Sodium contents of processed cheeses were about twice (1.0-1.2g/100g) the amount found in unprocessed hard cheeses. In unprocessed hard cheeses, the TP contents were 529-638 mg/100g, but the DP contents were lower (53-76% of TP). TP, DP, and sodium contents of cottage cheese were substantially lower than in processed or hard cheeses. Compared with milk, the TP content of cottage cheese was slightly higher, but the DP content was even lower. The results of dairy products are shown in Table 9.

Table 9. *Total and in vitro digestible P, protein, P-to-protein ratios, sodium contents, and P additives of the foods analysed.*

Product	TP/100g	DP/100g	Protein/100g	TP-to-Protein Ratio (mg/g)	DP-to-Protein Ratio (mg/g)	Na (g/100g)	P additives
Milk, 1.5% fat	108	85	3.2	33.8	26.6	0.04*	-
Skimmed milk	122	75	3.3	36.9	22.7	0.04*	-
Processed cheese, individually packed slices, 5% fat	574	589	23	24.9	25.6	1.1	E339, E452
Processed cheese, individually packed slices, 12% fat	647	720	24	27.0	30.0	1.2	E452, E339
Processed cheese, individually packed slices, 23% fat	584	576	21	27.8	27.4	1.1	E452, E339
Cheese spread, 9% fat	92	794	18	49.6	44.1	1	E452, E339
Cheese spread, 22% fat	755	772	19	39.7	40.6	1.1	E452, E339
Hard cheeses pool, 5-17% fat	638	484	30.3	21.1	16.0	0.54	-
Hard cheeses pool, 24-29% fat	529	282	26.5	20.0	10.6	0.56	-
Cottage cheese	146	71	13.8	10.6	5.1	0.3	-

*Protein and sodium contents based on Fineli®.

TP= total phosphorus; DP=*in vitro* digestible phosphorus

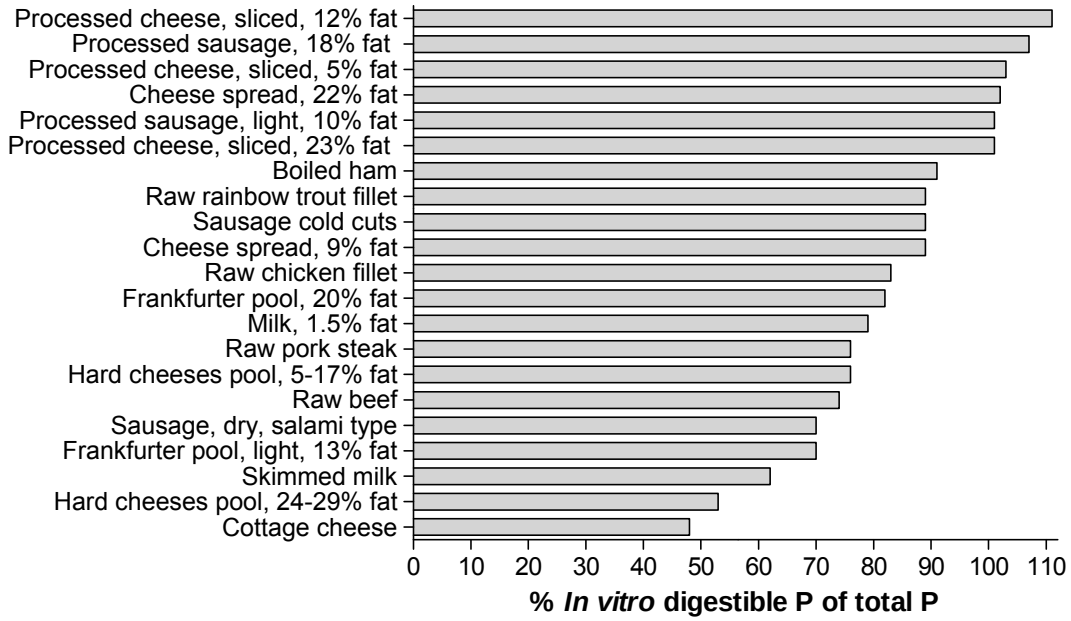

Figure 7 *Proportions of in vitro digestible P to total P among meat and dairy products.*

5.1.3 Plant foods

Of the breads analysed, the highest P content was found in rye crisp (TP 291 mg/100 g and DP 191 mg/100 g) due to its low water but high whole-grain content. Apart from the pooled crisp bread, the TP content of all other breads was similar. More variation relative to TP results was found in DP contents of breads. DP content of two very similar small rye breads containing potato differed, being 89 and 54 mg/100 g. High content of both TP (212 mg/100 g) and DP (201 mg/100 g) was also found in industrial muffins, which contain phosphate additives.

Practically all P in cola drinks and beer was soluble (10-16 mg/100 g in cola drinks; 22 mg/100 g in beer), but both DP and TP contents/100 g of the food were the lowest among the analysed foods. In legumes, seeds, and products prepared from legumes, TP content varied between 59 mg/100 g of soaked chickpea and 667 mg/100 g of sesame seed. Legumes contained an average DP content of 83 mg/100 g (38% of TP). The TP and DP contents and TP- and DP-to-protein ratios of plant foods analysed are presented in Table 10. Phytic acid contents of some of the analysed foods based on previously published data are also shown in the table. Proportions of DP to TP are shown in Figure 8.

Table 10. *Total and in vitro digestible P, protein, and phytic acid contents, and P-to-protein ratios of the analysed plant foods.*

Product	TP/100 g	DP/100g	Protein/100 g	TP-to-protein ratio (mg/g)	DP-to-protein ratio (mg/g)	Phytic acid g/100g
Rye bread pool	208	123	8.2c	25.4	15	0.3f
Rye crisp	291	191	10.1c	28.8	18.9	3.2/0.8/4.4f
Small rye bread containing potato 1	192	89	8.2c	23.5	10.9	Not available
Small rye bread containing potato 2	206	54	10c	20.6	5.4	Not available
Mixed grain bread with seeds	189	116	9.7c	19.5	12.0	Not available
Muffin pool[a]	212	201	6.6d	32.1	30.5	0.11g
Sweet bun pool	116	60	7.7c	15.1	7.8	0.8f
Cookie pool	125	43	6.1c	20.5	7.0	0.03-0.37g
Sesame seed (with hull)	667	42	26.9d	24.8	1.6	1.38h
Tofu (firm)	164	51	16.5c	9.9	3.1	0.84i (steamed, fried)
Green bean	57	24	1.9d	30.2	12.4	0.01i
Green pea (frozen)	118	50	5.1d	23.1	9.7	0.02i
Chickpea (soaked)	149	53	8.4d	17.7	6.3	0.5i(raw, unsoaked)
Red lentil	432	167	23.8e	18.2	7.0	0.26i
Green lentil	400	120	24.4d	16.4	4.9	0.49i
Pepsi Max[b]	14	15	<0.1 gc	Not applicable	Not applicable	Not available
Coca-Cola light[b]	13	13	0c	Not applicable	Not applicable	Not available
Coca-Cola[b]	19	16	0c	Not applicable	Not applicable	Not available
Cola pool[b]	17	16	<0.1 gd	Not applicable	Not applicable	Not available
Freeway Cola light[b]	11	10	<0.1 gd	Not applicable	Not applicable	Not available
Beer	21	22	0.4d	52.5	55	Not available

[a] Contains sodium polyphosphate as an additive
[b] Contains phosphoric acid as an additive
[c] Value provided by manufacturer
[d] Value based on National Institute for Health and Welfare (2011)
[e] Value based on Food Standards Agency (2002)
[f] Value based on Plaami & Kumpulainen (1995)
[g] Value based on Holland *et al.* (1988)
[h] Value based on Holland *et al.* (1992)
[I] Value based on Holland *et al.* (1991)

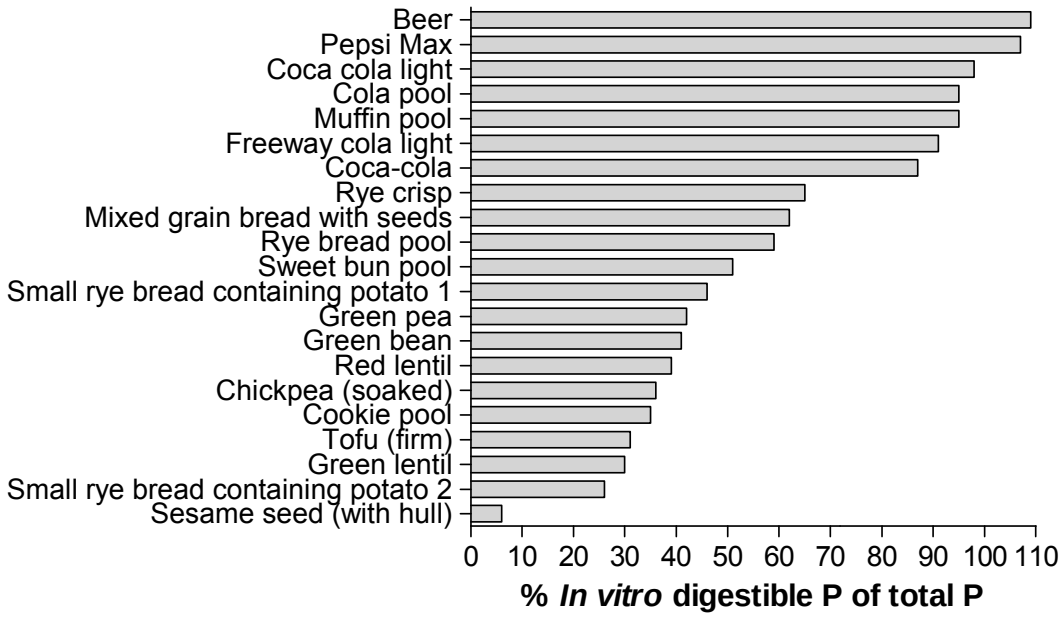

Figure 8 *Proportions of in vitro digestible P to total P among plant foods.*

5.1.4 Summary of results (Studies I and II)

When considering the highest and lowest P contents of all analysed foods, differences exist among TP and DP results. Tables 11 and 12 show the foods with the highest and the lowest TP and DP contents. Interestingly, all of the foods containing the highest absolute amounts of DP contained P additives.

Table 11. *Foods containing the highest amounts of TP and DP (Studies I and II).*

Food group	TP content (mg/100 g)	Food group	DP content (mg/100 g)
Processed and hard cheeses	529-892	Processed and hard cheeses	282-794
Sesame seeds	667	Boiled ham	255
Lentils	400-432	Processed sausages	224-242
Rye crisp	291	Muffin pool	201

Table 12. *Foods containing the lowest amounts of TP and DP (Studies I and II).*

Food group	TP content (mg/100 g)	Food group	DP content (mg/100 g)
Colas and beer	11-21	Colas and beer	10-22
Green bean	57	Green bean	24
Milk	108-122	Sesame seed	42
Sweet bun pool	116	Cookie pool	43
Green pea	118	Green pea*	50

*Several other plant foods with DP content 51-60 mg/100 g.

Tables 13 and 14 show the foods with the highest and the lowest TP- and DP-to-protein ratios. Among plant foods, all analysed foods, except beer, muffins, and rye crisp, had DP-to-protein ratios <15. Of foods of animal origin, only cottage cheese was among the foods with the lowest DP-to-protein ratios.

Table 13. *Foods containing the highest TP- and DP-to-protein ratios (Studies I and II).*

Food group	TP-to-protein ratio	Food group	DP-to-protein ratio
Beer	52.5	Beer	55
Cheese spread, 9% fat	49.6	Cheese spread, 9% fat	44.1
Cheese spread, 22% fat	39.7	Cheese spread, 22% fat	40.6
Muffin pool	32.1	Muffin pool	30.5
Green bean	30.2	Processed cheese, individually packed slices, 12% fat	30.0

Table 14. *Foods containing the lowest TP- and DP-to-protein ratios (Studies I and II).*

Food group	TP-to-protein ratio	Food group	DP-to-protein ratio
Raw beef	9.1	Sesame seed	1.6
Tofu (firm)	9.9	Tofu (firm)	3.1
Raw chicken fillet	10.0	Green lentil	4,9
Raw pork steak	10.1	Cottage cheese	5.1
Cottage cheese	10.6	Small rye bread containing potato 2	5.4

5.2 Human studies (Studies III and IV)

5.2.1 Habitual dietary intakes

Basic characteristics of the subjects and their mean habitual intakes of selected nutrients are shown in Table 15. The mean habitual P intake exceeded nearly 2.5-fold the recommendation for P in this age group (600 mg/d) (National Nutrition Council 2005). On average, Ca intake was also ample.

Table 15. *Background characteristics and mean habitual dietary intakes of subjects (SD) in Studies III and IV.*

Variable	Study III (n=16)	Study IV (n=12)
Age (years, range)	20-30	19-31
Body mass index (kg/m^2)	21.8 (2.2)	21.7 (1.6)
Energy intake (MJ/d)	7.5 (1.3)	7.3 (1.2)
Protein intake (g/d)	75 (24)	77 (26)
Phosphorus intake (mg/d)	1440 (406)	1580 (415)
Calcium intake (mg/d)	1030 (316)	1040 (320)

5.2.2 Serum phosphate and urinary phosphate excretion

In Study III, the S-Pi concentrations (AUC) differed between the study sessions (p= 0.0001, ANOVA) (Figure 9). The S-Pi concentration was elevated during the meat (p=0.0001), cheese (p=0.0001), whole-grain (p=0.006), and supplement (p=0.0001) sessions compared with the control session. Cheese increased the S-Pi concentration more than the other Pi sources (p=0.0001). Differences were present between study sessions in the S-Pi concentrations in the second-day fasting samples (p=0.0001, ANOVA). The S-Pi concentration remained higher in the cheese session on the following morning than in all other sessions (p=0.032-0.0001). Differences were found in U-Pi excretion between study sessions (p=0.0001, ANOVA) (Figure 9). U-Pi was higher in all Pi sessions than in the control session (p=0.001-0.0001). Meat increased U-Pi excretion more than grain (p=0.003) or cheese (p=0.005). The Pi supplement also increased U-Pi more than grain (p=0.001) or cheese (p=0.027).

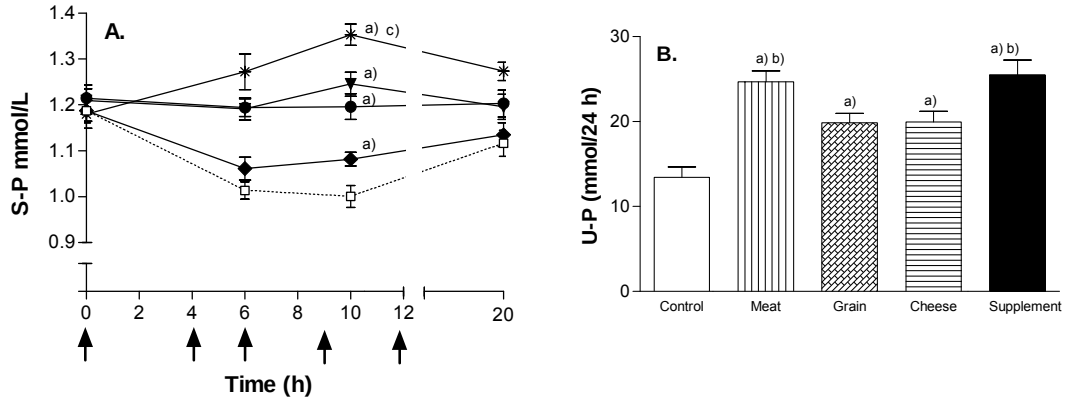

Figure 9 *Study III. Changes in serum phosphate (S-P) concentration (A) and 24-h urinary excretion of phosphate (U-P) (B) during the five study sessions. A: control (□), meat (●), supplement (▼), cheese (*), grain (♦. Arrows indicate mealtimes. The different phosphorus sources affected the AUC values of S-P (p=0.0001, ANOVA) and 24-h U-P (p=0.0001, ANOVA); [a]significantly different from control session, [b]significantly different from grain and cheese sessions, [c]significantly different from all other sessions.*

In Study IV, the S-Pi concentrations differed among the study sessions (p=0.0001, ANOVA) (Figure 10). The results of U-Pi were corrected for U-Crea excretion before the statistical analyses. Both MP (p=0.0001) and PP (p=0.0001) increased S-Pi compared with the control session. The difference between the MP and PP sessions in S-Pi was not statistically significant (p=0.18). Differences were found in the U-Pi excretion among the study sessions (p=0.0001, ANOVA) (Figure 10). Both MP and PP increased U-Pi compared with the control session (p=0.0001, both). No differences between the MP and PP sessions in U-Pi excretion were found (p=0.33).

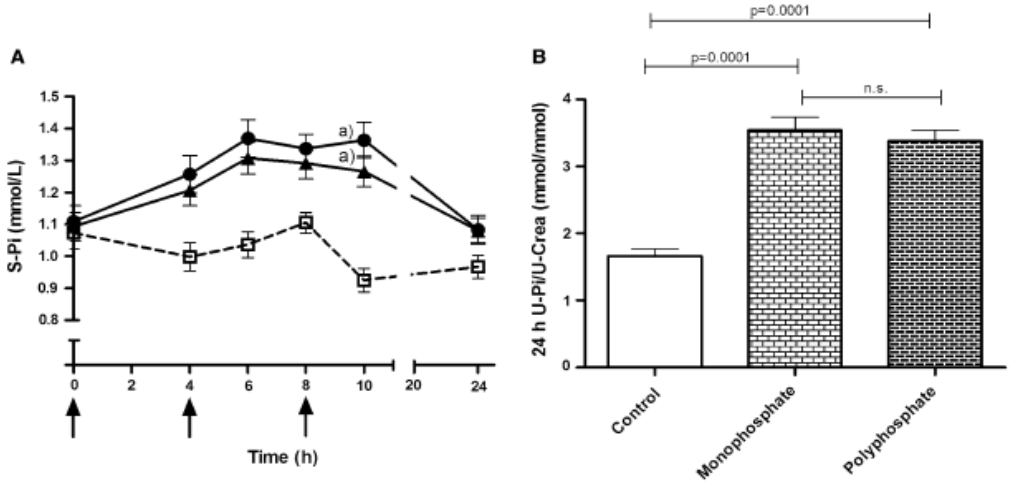

Figure 10 *Study IV. Changes in serum phosphate (S-Pi) concentration (A) and 24-h urinary excretion of phosphate (U-Pi/Crea) (B) during the three study sessions. A: control (□), monophosphate (●), polyphosphate (▲). The supplement administration times are indicated with an arrow. The supplements affected the area under the curve values of S-Pi (p=0.0001, ANOVA) and 24-h U-Pi/Crea (p=0.0001, ANOVA); [a]significantly different from control session.*

5.2.3 Serum ionized calcium and urinary calcium excretion

In Study III, the different foods affected the AUC of serum ionized calcium (S-iCa) (p=0.0001, ANOVA) and urinary calcium excretions (U-Ca) (p=0.0001, ANOVA) (Figure 11). The S-iCa concentration was greater in the cheese session due to high Ca intake than in the other sessions (p=0.0001). During the Pi supplement session, S-iCa concentration was lower than in the control session (p=0.027). Whole-grains (p=0.682) and meat (p=0.282) had no effect on S-iCa concentration relative to the control session. On the following morning, S-iCa concentration differed between study sessions (p=0.039, ANOVA). The 24-h morning fasting value was higher in the cheese session than in the supplement (p=0.018), whole-grain (p=0.023), or control (p=0.025) sessions and tended to be higher in the cheese session than in the meat session (p=0.050), but did not reach statistical significance. The U-Ca was higher during the cheese session than during all other sessions (p=0.0001) (Figure 11). In the meat session, the U-Ca was greater than in the Pi supplement session (p=0.003).

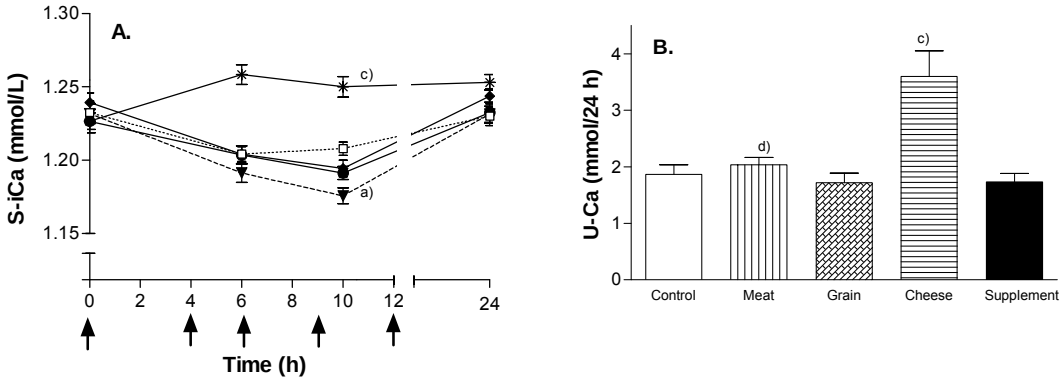

Figure 11 *Study III. Changes in serum ionized calcium (S-iCa) concentration (A) and 24-h urinary excretion of calcium (U-Ca) (B) during the five study sessions. A: control (□), meat (●), supplement (▼), cheese (*), grain (♦). Arrows indicate mealtimes. The different phosphorus sources affected the AUC values of S-iCa (p=0.0001, ANOVA) and 24-h U-Ca (p=0.0001, ANOVA); [a]significantly different from control session, [c]significantly different from all other sessions, [d]significantly different from phosphate supplement session.*

In Study IV, the S-iCa concentrations did not differ among study sessions (p=0.29, ANOVA) (Figure 12). The results of U-Ca were corrected for U-Crea excretion before the statistical analyses. The U-Ca excretion differed between the study sessions (p=0.001, ANOVA) (Figure 12). Only PP decreased the U-Ca in a statistically significant manner (p=0.002). However, the U-Ca excretion tended to decrease in the MP session relative to the control session (p=0.069). The PP decreased the U-Ca more than did the MP (p=0.014).

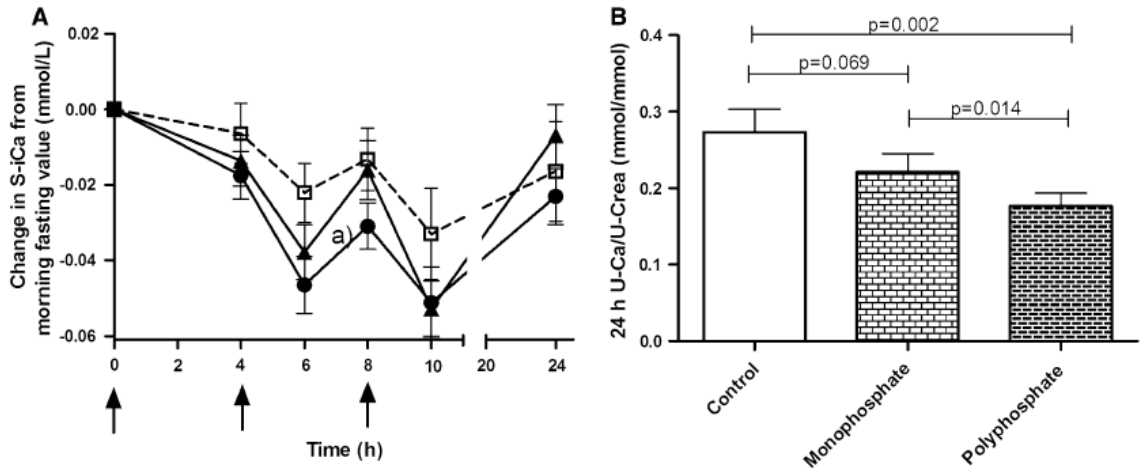

Figure 12 *Study IV. Changes in serum ionized calcium (S-iCa) concentration (A) and 24-h urinary excretion of calcium (U-Ca/Crea) (B) during the three study sessions. A: control (□), monophosphate (●), polyphosphate (▲). The supplement administration times are indicated with an arrow. The supplements did not affect the area under the curve values of S-iCa (p=0.29, ANOVA), but affected the 24-h U-Ca/Crea (p=0.001, ANOVA); [a]significantly different from control session.*

5.2.4 Serum parathyroid hormone concentration

In Study III, the AUC values of S-PTH differed between the study sessions (p=0.0001, ANOVA) (Figure 13). Compared with the other sessions, cheese decreased S-PTH most (p=0.0001). Pi supplement, by contrast, increased S-PTH concentration compared with the control session (p=0.039). Meat (p=0.346) or grain (p=0.498) had no effect on S-PTH. Differences were observed between study sessions in S-PTH concentrations of the second-day fasting samples (p=0.018, ANOVA). The S-PTH concentration was lower in the cheese session on the following morning than in all other sessions (p=0.048-0.004).

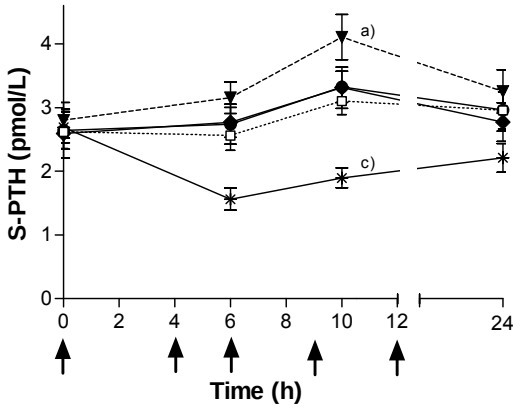

Figure 13 *Study III. Changes in serum parathyroid hormone (PTH) concentration during control (□), meat (●), supplement (▼), cheese (*), and grain (♦) sessions. Arrows indicate mealtimes. The different phosphorus sources affected the AUC values of S-PTH (p=0.0001, ANOVA); [a]significantly different from control session, [c]significantly different from all other sessions.*

In Study IV, the S-PTH concentrations differed between the study sessions (p=0.019, ANOVA) (Figure 14). Both MP (p=0.048) and PP (p=0.012) increased the S-PTH compared with the control session. The increase in S-PTH was equal with both Pi salts (p=0.74).

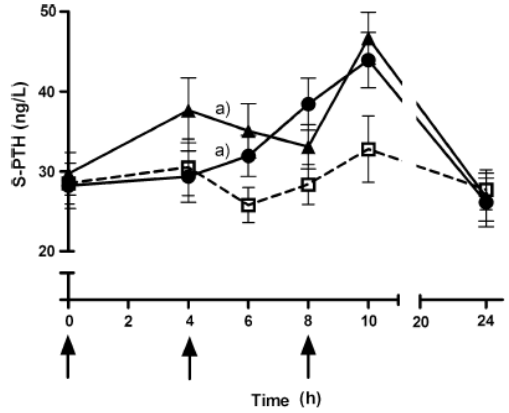

Figure 14 *Study IV. Changes in serum parathyroid hormone (S-PTH) concentration during the three study sessions. Control (□), monophosphate (●), polyphosphate (▲). The supplement administration times are indicated with an arrow. The supplements affected the AUC values of S-PTH (p=0.019, ANOVA); [a]significantly different from control session.*

5.2.5 Markers of bone metabolism (Study III)

Based on ANOVA, the AUC values of the activity of serum BALP (S-BALP) tended to differ among study sessions (p=0.094) (Figure 15), but did not reach statistical significance. In contrast analysis, the activity of S-BALP was higher in the meat session than in the control (p=0.045) or whole-grain (p=0.013) session. The second-day fasting values of S-BALP were not different among sessions (p=0.403, ANOVA).

Differences were present in urinary NTx excretions (U-NTx) among study sessions (p=0.0001, ANOVA) (Figure 15). The U-NTx was higher in the meat session (p=0.049) than in the control session. U-NTx excretion tended to increase in the whole-grain (p= 0.053) and Pi supplement (p=0.083) sessions compared with the control session, but did not reach statistical significance. The NTx excretion was lower during the cheese session than during the control session (p=0.008). The NTx results are shown as bone collagen equivalents (nmol BCE) without creatinine corrections due to the differences in U-Crea results among the study sessions.

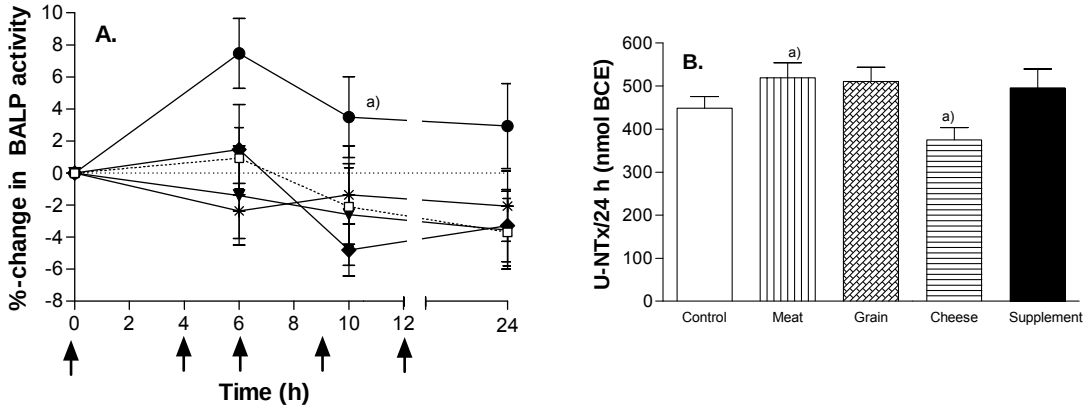

Figure 15 *Study III. Changes in serum BALP (S-BALP) activity from the morning fasting value (A) and 24-hour urinary excretion of NTx (U-NTx) (B) during the five study sessions. A: control (□), meat (●), supplement (▼), cheese (*), grain (♦). Arrows indicate mealtimes. The different phosphorus sources tended to affect the AUC values of S-BALP (p=0.094, ANOVA) and affected U-NTx (p=0.0001, ANOVA); [a]significantly different from control session.*

5.2.6 Serum creatinine and urinary creatinine excretion (Study III)

In Study III, the AUC values of serum creatinine (S-Crea) concentrations (p=0.0001, ANOVA) and urinary excretion of creatinine (U-Crea) differed among study sessions (p=0.0001, ANOVA) (Figure 16). Because meat has a high creatinine content, the S-Crea concentration was higher in the meat session than any of the other sessions (p=0.0001). The S-Crea concentration was higher in the cheese session than in the control session

(p=0.038). The U-Crea was greater in the meat session than in all other sessions (p=0.021-0.0001) (Figure 16). Also, in the cheese session, the U-Crea was higher than in the control (p=0.030) or whole-grain (p=0.019) sessions. Due to the differences in U-Crea, the results of U-Pi, U-Ca, and U-NTx were not corrected for U-Crea, as this would have skewed the results. In Study IV, no differences were found in S-Crea and U-Crea (data not shown).

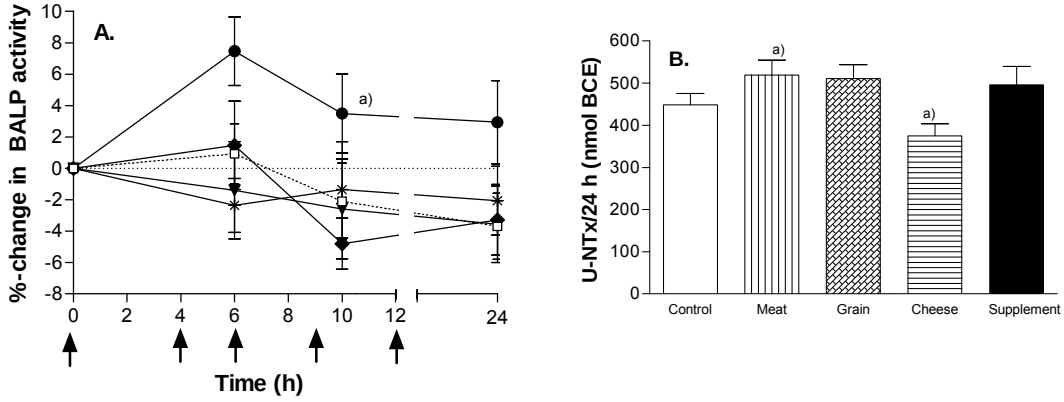

Figure 16 *Study III. Changes in serum creatinine (S-Crea) concentration (A) and 24-h urinary excretion of creatinine (U-Crea) (B) during the five study sessions. A: control (□), meat (●), supplement (▼), cheese (*),grain (♦). Arrows indicate mealtimes. The different phosphorus sources affected the AUC values of S-Crea (p=0.0001, ANOVA) and 24-h U-Crea (p=0.0001, ANOVA); [a]significantly different from control session, [c]significantly different from all other sessions.*

6 Discussion

6.1 Phosphorus content of foods

6.1.1 Meat and meat products

Of the foods analysed, raw meats and meat products were not among those with the highest TP contents, but the DP contents of some processed meats were among the highest (Table 11). When raw meats were compared, only small differences were found among chicken, pork, beef, and rainbow trout. Our TP results of these P additive–free meats are similar to those of unenhanced meats published previously (Sullivan *et al.* 2007, Sherman & Mehta 2009b). Compared with those of sausages and cold cuts, the P-to-protein ratios and sodium contents of the unenhanced meats are lower, hence more beneficial to CKD patients. The DP contents of raw meats varied more than the TP contents, the lowest DP content being found in beef and the highest in rainbow trout. However, TP and DP contents of cooked meats should be analysed, as cooking itself and likely also the cooking method affect the P content of a food (Cupisti *et al.* 2006, Delgado-Andrade *et al.* 2011).

In sausages and cold cuts, the lowest percentages of DP to TP were found in the salami-type sausage and in low-fat frankfurters. The content of P additives in the salami-type sausage was likely lower than that in other sausages, as one of the sausages in the salami-type sausage pool did not contain any P additives. Also, the consistency of salami is different from that of other sausages. Phosphates increase the water content of a product, which is an unwanted quality in a salami-type sausage. Due to its high protein content, the P-to-protein ratios of the salami-type sausage were surprisingly low; by contrast, the sodium content of the salami-type sausage was the highest among the foods of this study. Our results indicate that frankfurters may contain less P than other sausages, but other brands should be analysed before drawing further conclusions. Also, sausages prepared without P additives should be analysed for comparisons with current products. As the market shares of P additive–free sausages are small (at least in Finland), these products were not analysed here.

6.1.2 Dairy products

All processed and hard cheeses had the highest TP and DP contents among all foods analyzed. Our results confirm previous data on cheeses as P-rich foods, as an extremely high content of DP was found in processed cheeses. In Study III, fermented cheese (not containing P additives) increased the S-Pi concentration more than meat, whole-grains, or even a mixture of P additives, suggesting that P from cheese is easily absorbed.

Furthermore, in a cross-sectional study on a randomly selected subgroup of 31- to 43-year-old Finnish women, consumption of processed cheeses was associated with a higher mean S-PTH concentration (Kemi *et al.* 2009).

Variation in both DP (589 to 794 mg/100 g) and TP (574 to 892 mg/100 g) can be found among processed cheeses. The P contents seem not to depend on the fat or protein content of the processed cheese. However, in cheese spreads, the TP and DP contents seem to be higher than in sliced processed cheese. Possibly, the softer texture of cheese spreads requires more P additives than the harder texture of sliced, processed cheeses. The sodium contents in processed cheeses are substantially higher than those in other types of cheeses; part of the sodium is from the sodium phosphate additives. It is notable that the use of sodium phosphate-containing processed cheeses may substantially increase not only P intake but also sodium intake. Furthermore, the P-to-protein ratios of processed cheeses are extremely high (Table 13), thus further confirming that they are unsuitable in the diet of CKD patients.

Contrary to other cheeses, cottage cheese had the lowest absolute DP content and DP-to-TP proportion among the milk and meat products, and one of the lowest TP- and DP-to protein ratios among all foods analysed. The P contents of fresh cheeses are lower than those of hard cheeses or processed cheeses because of the higher water content and different preparation technique of fresh cheeses. When considering the diets of CKD patients, due to the low P-to-protein ratio and sodium content, cottage cheese is clearly more beneficial than hard or processed cheeses.

6.1.3 Plant foods

Of the foods analysed in this study, the proportion of DP was lowest in legumes and seeds (6-42% of TP). Sesame seed was among the foods with the highest TP content, but also among the foods with the lowest DP content, which could mean that at least some seeds are poor P sources. If confirmed in further analyses, this result would indicate that whole sesame seeds, possibly some other seeds as well, could be used in a P-restricted diet. In a short-term controlled study, sesame seeds did not increase S-Pi relative to the control session (Kärkkäinen *et al.* 1997), which is in line with the present results.

Earlier, it has been suggested that only 25% of P from beans actually gets absorbed (Uribarri 2007). Even though our results of DP in legumes are on average higher than this, they support the opinion presented recently (Kalantar-Zadeh *et al.* 2010) on the use of legumes in patients with CKD: very strict limitations appear to be unnecessary. Further, our results are in line with those of a crossover trial comparing the effects of vegetarian and meat protein sources on P homeostasis in CKD (Moe *et al.* 2011). The cooking time of lentils, green beans, and peas is relatively short, and these legumes do not require soaking before cooking. A large amount of phytic acid is therefore unlikely to be hydrolysed when

cooking these legumes. Thus, the DP content in lentils, green beans, and peas is anticipated to not increase notably when these products are cooked. However, further analyses on cooked legumes are required to evaluate P absorption, especially for those requiring a longer cooking period such as chickpeas and several beans. Analyses on phytic acid content of differently processed legumes, seeds, and grain products could also improve knowledge of P availability.

In cola drinks and beer, all P was digestible. This result is in accordance with past information on absorbability of P from food additives because all P in cola drinks is from phosphoric acid, which is likely to degrade efficiently in the intestine. TP content in cola drinks was similar to the values published earlier (Murphy-Gutekunst 2005). In beer, P is from the grain used in preparing beer; in Finland, this is most often barley. On the basis of these results, it seems that during malting and other processing of grain when preparing beer, P is efficiently released from the grain in a highly digestible form, resulting in even higher P content than in cola beverages.

DP content of different rye breads may vary substantially, likely depending on the amount and proportion of rye flour and crushed rye grain, the content of other grains, the use of yeast, and the length of fermentation. White bread, the P content of which is known to be lower than that of whole-grain breads, was not analysed. According to our previous analyses using the same method, we found that DP content of white bread is also lower than that of rye or mixed-grain bread. However, based on these results, a rye bread with a lower P bioavailability than in the currently available rye breads could potentially be developed. The possibility of using rye bread to some extent in a P-restricted diet would likely improve compliance with the diet in countries where rye bread is a staple food. Furthermore, whole-grain consumption is associated with a lower risk of cardiovascular diseases and mortality (Jacobs *et al.* 2000, Liu *et al.* 2000).

When considering sweet bakery products, buns (leavened with yeast) and cookies (P additive-free) have a P content similar to that in white bread. A striking difference to other wheat products was, however, found in industrially prepared muffins containing a P additive (baking powder). TP content of muffins was similar to that of rye bread, but DP content was higher than in any breads. Therefore, individuals consuming large amounts of bakery products with a P-containing baking powder may have an elevated intake of highly bioavailable P, suggesting that bakery products can be another important source of DP in addition to meat products, processed cheeses, and carbonated beverages.

6.1.4 Conclusions about the phosphorus content of foods

Both TP and DP contents varied widely between the different food groups analysed and also within many food groups. Results of TP can largely be explained by protein content of the food and/or use of P-containing food additives. Proportion of DP to TP content

seems to be affected by processing of the food in phytic acid-containing plant foods and by the presence of P-containing food additives. Furthermore, our results support previous findings of better P absorbability in foods of animal origin. Variation among similar products does, however, exist. This variation often depends not only on the protein or fat content of the food but also on the use of P additives, which is affected by the desired texture of the food.

In conclusion, the most beneficial P sources for CKD patients and for any other persons wishing to reduce their P intake could be legumes (and products prepared from them, e.g. tofu), fresh cheeses, and whole seeds. Of foods of animal origin,‘‘natural’’ unenhanced meats are a better choice in terms of P-to-protein ratios and sodium contents than processed or hard cheeses, sausages, and cold cuts. More research is, however, needed on the P contents of different foods and the absorbability of P from different foods with an *in vivo* method.

6.2 Dietary interventions

6.2.1 Effects of P sources on serum and urinary phosphate concentration as indicators of bioavailability

Serum and urinary Pi concentrations are indirect measures of absorbed P, and U-Pi is affected by PTH secretion. Still, in a controlled setting, comparing S- and U-Pi during different sessions provides information on P absorbability. Based on S-Pi and U-Pi in Study III, P from meat and supplements appears to absorb better than P from whole-grains. The whole-grain foods given to the subjects (oatmeal porridge, rye bread) were unfermented. Because fermentation can enhance the absorption of minerals in phytate-containing foods, the bioavailability of P may be greater from fermented whole-grains and even greater from low-fibre grains. However, in Studies I and II the DP content seemed to be higher in cheese and meat than in grains and legumes. The P supplements and meat had similar effects on S-Pi and U-Pi. As meat did not affect S-PTH, more research is needed to clarify whether this difference is due to variation in absorbability, protein intake, or another factor.

Estimation of the absorbability of P from cheese is more complex than that of whole-grains because the high Ca content decreases PTH secretion, which in turn decreases U-Pi. Hence, the low U-Pi excretion in the cheese session was probably due to low S-PTH concentration caused by high Ca intake. S-Pi increased during the cheese session more than during any other session. Decreased U-Pi caused by low PTH secretion may have caused an increase in S-Pi by elevating the renal P threshold. At the same time, a decrease in bone resorption (as indicated by lowered NTx excretion) likely caused a drop in the

release of P from bone, which could decrease S-Pi concentration. The effects of these two mechanisms on S-Pi are, therefore, the opposite. P intake during the cheese session (1624 mg) was slightly higher than during other P sessions, which may also have increased S-Pi concentration. In addition, the high Ca intake (290-602 mg/meal) may have partly inhibited the absorption of P in the intestine. Based on this study, we do not know whether this inhibition occurred. Probably many of these factors simultaneously affected S-Pi in the cheese session, the outcome being increased S-Pi. However, based on another short-term controlled study, both milk and cheese increase S-Pi efficiently, suggesting that P from dairy foods is well absorbed (Kärkkäinen *et al.* 1997).

In Study IV, both Pi salts, commonly used in the food industry, affected S-Pi and U-Pi in a similar manner, and therefore, were likely to be absorbed equally well.

6.2.2 Serum ionized calcium and urinary calcium excretion

In Study III, the increase in S-iCa and U-Ca during the cheese session was expected due to the high Ca intake. Only the P supplement decreased S-iCa, which is in line with the increase in S-PTH during the P supplement session. Interestingly, meat did not affect S-iCa despite similar increases in S-Pi and U-Pi.

U-Ca excretion during the meat session was higher than during the P supplement session, but did not differ from the control session. Earlier, in a 10- to 15-day study, addition of meat to a diet low in protein, Ca, and P was observed to increase U-Ca (Schuette & Linkswiler 1982). The rise in U-Ca in response to increased dietary protein seems to be mostly due to enhanced intestinal Ca absorption (Kerstetter *et al.* 2005). It is possible that the increase in U-Ca in the P supplement session relative to the control session went unnoticed in our study, e.g. due to incomplete urine collections, or the magnitude of the increase was so small that we were unable to detect it. However, the difference in U-Ca during the meat and P supplement sessions is logical, as increased protein intake of the meat session is expected to increase U-Ca, and the increased S-PTH during the supplement session decreases U-Ca.

In Study IV, the only significant difference between the MP and PP sessions was found in U-Ca, which PP decreased more than did MP. As a consequence of the increased level of S-PTH in both Pi sessions, a decrease in U-Ca in both sessions would have been expected. We consider it likely that MP ingestion also decreases U-Ca, even though in this study the decrease relative to the control session reached only the level of a trend.

The larger decrease in U-Ca in the PP session than in the MP session was likely due to PP binding more Ca in the intestine than did MP (van Wazer & Callis 1958); hence, less Ca was absorbed in the PP session than in the MP session. To verify this conclusion, faecal collections would have been necessary. However, also in the study of Zemel and

Linkswiler (1981) in young men, the authors concluded that the greater Ca loss with the PP diet was due to a decrease in Ca absorption. In their study, no difference in U-Ca excretion between the MP and PP diets was observed, in contrast to our results. Based on differences in cAMP excretion (indicating differences in PTH secretion), the differences in U-Ca would have been expected since Ca intake was the same during both diets. In our study, two mechanisms may have contributed to the difference in U-Ca between the MP and PP sessions: reduced intestinal Ca absorption itself and possibly also increased PTH secretion, although we noted no statistical differences in S-PTH between the MP and PP sessions. In the study of Zemel and Linkswiler (1981), potassium (K) intakes differed between the MP and PP diets because the MP salt was a monobasic potassium phosphate and the PP salt was a sodium phosphate. The difference in K intake may explain the U-Ca results in their study since K increases the retention of Ca (Lemann *et al.* 1989).

The MP and PP supplements did not affect S-iCa, even though in Study III and in previous similar intervention studies (Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006) similar amounts of P decreased S-iCa. Technical difficulties in performing the S-iCa probably affected the results.

6.2.3 Parathyroid hormone

Based on Studies III and IV, cheese decreases S-PTH due to its high Ca content, meat and grain have no effect on S-PTH, and P additives – both mono- and polyphosphates – increase S-PTH relative to the control session.

The effect on mineral metabolism of P additives apparently differs from that of foods containing P naturally. In most interventions, P has been administered as a supplement (Portale *et al.* 1986, Calvo & Heath 1988, Silverberg *et al.* 1989, Brixen *et al.* 1992, Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006). In all of these studies, a rise in PTH secretion as a result of P administration was observed. At least two interventions have been conducted using diets assembled from common foods (Calvo *et al.* 1988, Calvo *et al.* 1990,). Also, in these studies a rise in S-PTH concentration with a low-Ca, high-P diet was found. However, the intake of P additives was probably rather high because the high-P test diet included several P additive-containing foods. For this reason, the effects of the high-P diets can be due to P additives in these studies, too. Moreover, Bell *et al.* (1977) observed enhanced parathyroid activity as a response to foods containing P additives compared with the same foods prepared without these additives. The PTH-rising effect of P additives compared with other sources could be due to their better absorbability.

In Study IV, both Pi salts (MP and PP) increased S-PTH, which is in line with previous intervention studies conducted with P additives (Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006). Hence, both MP and PP affect PTH secretion similarly, even though previously MP increased U-cAMP (as a marker of PTH secretion) more than did PP

(Zemel & Linkswiler 1981). Since cAMP excretion is an indirect measure of PTH secretion, we consider our results more accurate. The rise in S-PTH may partly have been due to the Ca-binding capacity of Pi in the gut, especially PP. In the present study, however, we were unable to detect a difference in S-PTH between the MP and PP sessions. Possibly,t more samples would have been required to detect all of the differences in the serum measurements. As no samples were taken between 00:00–4:00 and 10:00–24:00 hours, we do not know what happened with S-PTH, S-Pi, and S-Ca during these periods. With more frequent sampling, we also would have seen the diurnal variation of PTH and other serum measurements in more detail.

Na intake may also increase PTH secretion by increasing Ca excretion (Evans *et al.* 1997). Na intake may increase U-Ca excretion, which could lead to a need to maintain S-Ca by increasing PTH secretion. Kärkkäinen & Lamberg-Allardt (1996) previously showed that a dose of 1.4 g Na as NaCl does not affect PTH secretion. In Study IV, the difference in Na intakes was highest (1.8 g) between the PP and control sessions, the differences between the control and MP sessions and the MP and PP sessions being 1.1 and 0.7 g, respectively. Theoretically, Na intake could have contributed to the S-PTH increase in the PP session. In that case, higher Ca excretion would have been expected. However, since Ca excretion was lowest in the PP session, we consider it unlikely that the difference in Na intakes would have explained the rise in S-PTH in the Pi sessions.

6.2.4 Markers of bone metabolism (Study III)

Urinary NTx excretion tended to increase in the P supplement session compared with the control session, implying greater bone resorption as a result of increased PTH secretion. Calvo *et al.* (1988) observed increased excretion of hydroxyproline, another marker of bone resorption, with a low-Ca, high-P test diet compared with the control diet (820 mg Ca, 930 mg P) in their 8-day study, but in their 4-week study, no difference in the excretion of hydroxyproline between the low-Ca, high-P test diet and control diet (800 mg Ca, 900 mg P) was found (Calvo *et al.* 1990). In two other studies, high P diets had no effect on the concentrations of resorption markers (Kärkkäinen & Lamberg-Allardt 1996). In the short-term study of Kemi *et al.* (2006), urinary excretion of NTx increased acutely as a response to 1500 mg of P administered as an inorganic P additive. Therefore, the results of NTx in the supplement session are in accordance with this previous study. Urinary NTx may respond to oral P load more rapidly than other resorption markers used in older studies. The differences in protocols of the P supplementation studies and sensitivities of different resorption markers probably explain the differences in results.

Earlier, an acute decrease in serum BALP activity after administration of 1500 mg (Kärkkäinen & Lamberg-Allardt 1996, Kemi *et al.* 2006) and 750 mg (Kemi *et al.* 2006) of inorganic P was observed. In the present study, by contrast, the P supplement had no effect on serum BALP. This could be due to the timing and number of blood samples;

when the 14:00-hour blood sample (the first blood sample after the fasting sample) was taken, the subjects had only ingested ca. 500 mg of P from the supplement. The 18:00-hour blood sample was taken immediately after the last P supplement dose. As the next blood sample was taken the following morning, we do not know whether serum BALP activity had decreased in the evening after 18:00 hours.

During the meat session both bone formation and resorption increased, which may be due to the high protein intake from meat. The average protein intake in the meat session was 2.1 g/kg body weight, which comprised 25% of total energy. Because protein consumption increases urinary Ca excretion, high protein intake has been speculated to be detrimental to bone health (Kerstetter *et al.* 2003). The effect of protein intake on bone seems, however, to depend on the protein source as well as on other nutrients in the food (Massey 2003).

The increase in both BALP and NTx probably indicates increased turnover of bone during the meat session. The effects of meat proteins and meat phosphates on bone should be confirmed in a longer term study. Alternatively, some other substance in meat might have caused the increases in the markers of bone metabolism during the meat session. Our result is contrary to that of Kerstetter *et al.* (2005), who observed a non-significant trend towards a reduction in the rate of bone turnover with a high-protein diet.

The reason for increased NTx excretion during the whole-grain session remains obscure. Possibly, the high intake of phytates from unfermented whole-grain inhibited Ca absorption, which could have caused an increase in NTx excretion.

6.2.5 Conclusions about the dietary interventions

In conclusion, we observed marked differences in metabolic responses to different foods and P additives. The effects of high P intake appear to depend on the P sources consumed and other nutrients of the P-containing food. P additives commonly used by the food industry seem to be absorbed well and increase S-PTH, as shown in previous studies. By contrast, P from meat or whole-grains appears not to affect S-PTH. Because of the high Ca content, cheese affects bone metabolism favourably. Meat increased bone turnover, possibly because of its high protein content. PP-containing products could more likely interfere with Ca balance than MP-containing foods due to the Ca-binding property of the PP compound. Longer term intervention studies are needed to confirm our results.

6.3 Strengths and limitations of the studies

6.3.1 Phosphorus analyses of foods

In Studies I and II, the sampling was performed carefully to ensure the representativeness of the analyses. By analysing the nationally most popular products in each food category, we believe the results are nutritionally relevant. For each analysis, products from different batches were bought to exclude the effect of possible variation in nutrient contents between batches. Due to the sampling procedure, the representativeness of the results of the food studies can be considered better than in several other studies where P contents have been measured. Reference materials were used in every batch analysed to appropriately report the accuracy and precision of the both TP and DP analyses. These data are lacking in most other P analyses published. The recent papers on food P contents have concentrated on meats and meat products. We have extended the analyses to foods of plant origin, which can be considered another strength of these studies.

The TP contents of the foods were analysed by a routine method, and the results are in accord with previously published findings. The analysis of DP is a novel method, the use of which provides additional information to the usual TP analyses. The results for DP were mostly consistent with previous data on P availability, but they should be confirmed with another, *in vivo* method. In some samples with extremely high DP proportion, the DP content slightly exceeded the TP content, which may indicate that the method of DP analysis is not equally accurate for all samples. Considering the importance of most P sources also as protein sources data analysis of the protein content of the foods would have provided more accurate P-to-protein ratios.

6.3.2 Dietary interventions

The dietary interventions of this thesis were short-term studies in which the nutrient intakes were completely controlled. Based on previous research (Kemi *et al.* 2006, Kärkkäinen & Lamberg-Allardt 1996), the study design is suitable for comparing the effects of different P sources. However, as discussed earlier, more frequent sampling would have revealed the differences among different sessions in more detail – some of the differences may have remained unseen due to the sampling schedules. An increase in the number of samples would have required the use of cannulas (instead of repetitive punctures), which would have necessitated that the subjects stay at the research unit throughout the sessions.

The short follow-up period is a limitation of the studies. Longer term intervention studies are needed to confirm our results. However, for comparing absorbability of P from

different sources, we believe the 24-h study periods were adequate, as most P is absorbed passively.

Problems in designing diets with similar amounts of nutrients using different foods is another limitation of the studies. In Study III, the whole food approach can hence be considered a limitation. When feeding meat, whole-grains, and cheese in separate sessions, the intakes of protein, fibre, and Ca varies among these sessions. We considered the possibility of equalizing the intakes of these nutrients by using supplements. However, if supplemented, the test diets would not be realistic in representing the nutrient content of the P sources. Furthermore, the absorbability of a nutrient could be different between foods and dietary supplements. For this reason, a fibre supplement, for instance, should have contained identical fibres as the whole-grain foods in the test diet. Such a supplement would also have increased P intake as a large part of whole-grain P can be found in the fibre part of a grain. In Study IV, the differences in Na intakes between the sessions should have been corrected by adding a Na salt to the diet during monophosphate and control sessions, even though it is unlikely that the difference in Na intakes affected S-PTH (Kärkkäinen & Lamberg-Allardt 1996).

The main outcome variable in both studies was S-PTH. According to power calculation based on S-PTH (assuming 80% power with α=0.05), a sample size of 13 was adequate (Kemi *et al.* 2006). However, as intra- and interindividual variation is greater in bone formation and resorption markers than in S-PTH, a larger sample size might have better revealed differences in these markers, especially considering that the P intakes were practically the same during different sessions.

Due to differences in mineral metabolism between men and women (Calvo *et al.* 1990) and between pre- and postmenopausal women (Renier *et al.* 1992), we focused on one age group and gender. This impedes generalization of our results to men and elderly women. Thus, in addition to longer term studies on P sources, also comparisons in different subject groups, such as in younger and elderly patients and in healthy individuals and CKD patients, are needed in the future.

Ideally, measuring more than one bone resorption and formation marker would be optimal, as different markers depict different stages of bone metabolism. Furthermore, measuring markers of bone metabolism in Study IV would have been interesting. It is unsure whether FGF-23 responds to P loads acutely, but measuring its concentrations would at least have provided more information on the inter- and intraindividual changes of this marker in healthy young women. Due to budget constraints, additional measurements were not possible.

7 Summary and conclusions

This thesis provides new information on P content of foods, P absorbability, and the effects of different P sources on mineral metabolism. Based on these studies, P is absorbed with different efficacy from different foods, and effects of P on health may depend on the source from which it is ingested. The latter is explained by differences in absorbability of P, but also by other nutrients that each P source contains. Inorganic P used as an additive is absorbed more efficiently than P from natural sources and affects mineral metabolism negatively. In addition, inorganic P additives often contain Na, which has a negative effect on cardiovascular and bone health.

Specific findings of each study were as follows:

Study I: Differences existed in total and *in vitro* digestible P contents among commonly used plant foods and beverages. The highest proportions of DP (87-100%) were found in cola drinks, beer, and P additive-containing muffins, the lowest in sesame seeds (6%) and legumes (30-42%). In grain products, the DP proportion varied (26-65%) likely depending on the grains used, the presence of yeast, and other techonological properties such as length of fermentation. Base on this study, legumes and seeds may be relatively poor P sources despite high TP content.

Study II: Differences existed in total and *in vitro* digestible P content of commonly used meat and milk products. The highest proportions of DP (70-100%) were found in P additive-containing processed cheeses and meat products, the lowest in cottage cheese (48%). Chicken, pork, beef, and rainbow trout contained similar amounts of TP (199-232 mg/100 g), but slightly more variation was found in their DP contents (147-207 mg/100 g). Based on these results, variation in both TP and DP contents of similar products can be substantial. Generally, foods of animal origin seem to have higher DP amounts and proportions than plant foods.

Study III: In the short-term controlled intervention study, different P sources affected markers of P, Ca, and bone metabolism differently in healthy young females. Only a P supplement containing inorganic P additives increased S-PTH. Meat increased both bone formation and bone resorption. Cheese decreased S-PTH and bone resorption, indicating a favourable change in bone metabolism. Based on S-Pi and U-Pi, P from unfermented whole-grains seems not to be absorbed as efficiently as P from other sources used in this study.

Study IV: In the short-term controlled intervention study, mono- and polyphosphates (MP and PP, respectively) affected markers of P and Ca metabolism similarly in healthy young females. Both MP and PP increased S-PTH, which, in the long run, may have a negative

effect on bone. PP decreased U-Ca more than did MP, implying that PP may bind Ca in the intestine more than MP.

The results of this thesis suggest that when evaluating effects of P intake on health, the P sources should be scrutinized. More research is, however, needed on absorbability of P, for example, from differently prepared legumes and grains, but longer term studies on the effects of P intake on cardiovascular and bone health are also warranted.

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Appendix I

Table 1. *Phosphorus contents of selected raw, lean and boneless meats.*

Meat	P content mg / 100 g
Beef	210[1]
Chicken	160[1] / 210[2]
Lamb	150[1]
Moose	220[1]
Pork	160[1] / 210[2]
Reindeer	310[1]

[1]Source: National Food Database Fineli®, National Institute for Health and Welfare
[2]Source: Sherman & Mehta 2009

Table 2. *Phosphorus content of selected plant-based foods.*

Food	P content mg / 100 g
Soy meal	839
Sunflowerseed	700
Almond	490
Lentils green/brown	350
Dark chocolate	200
Boiled chick pea	168
Boiled sweet corn	85
Tomato	30
Apple (with skin)	8

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

Appendix II

Table 1. *Phosphate-containing food additives and their E codes and group names.*

E code	Additive	Group name of additive
E338	Phosphoric acid	Ortho phosphates
E339	Monosodium phosphate Disodium phosphate Trisodium phosphate	
E340	Monopotassium phosphate Dipotassium phosphate Tripotassium phosphate	
E341	Monocalcium phosphate Dicalcium phosphate Tricalcium phosphate	
E343	Monomagnesium phosphate Dimagnesium phosphate	Magnesium phosphates
E450	Disodium diphosphate Trisodium diphosphate Tetrasodium diphosphate Dipotassium diphosphate Tetrapotassium diphosphate Dicalcium diphosphate Monocalcium diphosphate	Diphosphates = Pyrophosphates
E 451	Pentasodium triphosphate Pentapotassium triphosphate	Tripolyphosphates
E 452	Sodium polyphosphate Potassium polyphosphate Sodium-calcium polyphosphate Calcium polyphosphate	Polyphosphates
E 541	Sodium aluminium phosphate	Aluminium phosphate
E1410	Monostarch phosphate	Starch phosphates (Organic P additives)
E1412	Distarch phosphate	
E1413	Phosphorylated distarch phosphate	
E1414	Acetylated distarch phosphate	

Modified from Suurseppä *et al.* (2001)