Coffee Consumption in Relation to Osteoporosis and Fractures

Observational Studies in Men and Women

HELENA HALLSTRÖM
Dissertation presented at Uppsala University to be publicly examined in Sal IX, Universitetshuset, Biskopsgatan 3, Uppsala, Friday, April 26, 2013 at 09:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

During the past decades, the incidence of osteoporotic fractures has increased dramatically in the Western world. Consumption of coffee and intake of caffeine have in some studies been found to be associated with increased risk of osteoporotic fractures, but overall results from previous research are inconsistent. Despite weak evidence, some osteoporosis organisations recommend limiting daily coffee or caffeine intake.

The primary aim of this thesis was to study the association between long-term consumption of coffee and bone mineral density (BMD), incidence of osteoporosis and fractures. A secondary aim was to study the relation between tea consumption and fracture risk.

An increased risk of osteoporotic fractures in individuals who consumed ≥ 4 cups of coffee vs < 1 cup coffee per day was demonstrated in a study of 31,257 Swedish middle-aged and elderly women (a part of the Swedish Mammography Cohort - SMC) when calcium intake was low (< 700 mg/day). However, no higher risks of osteoporosis or fractures were observed in the full SMC with increasing coffee consumption. In the full SMC (n = 61,433) the follow-up was longer and the number of fractures was higher. Similarly, no statistically significant associations between consumption of coffee (≥ 4 cups of coffee vs < 1 cup) and incidence of osteoporotic fractures were observed in the Cohort of Swedish Men (COSM), including 45,339 men. Calcium intake did not modify the results from the investigations performed in the full SMC or COSM.

Nonetheless, a 2 - 4% lower BMD at measured sites was observed in men participating in the PIVUS cohort and in women from a sub-cohort of the SMC who consumed ≥ 4 cups of coffee vs < 1 cup daily. Individuals with high coffee intake and rapid metabolism of caffeine had lower BMD at the femoral neck.

No association between tea consumption and risk of fractures was found in the studies.

In conclusion, the findings presented in this thesis demonstrate that high consumption of coffee may be associated with a modest decrease in BMD. However, there was no evidence of a substantially increased incidence of osteoporosis or fractures typically associated with osteoporosis.

Keywords: Coffee, Tea, Caffeine, Bone mineral density, Osteoporosis, Fractures, Cohort studies

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To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV Hallström H., Wolk A., Glynn A., Michaëlsson K. and Byberg L. Coffee consumption and risk of fracture in a prospective longitudinal cohort of Swedish men (COSM). In manuscript.

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## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>9</td>
</tr>
<tr>
<td>Background</td>
<td>11</td>
</tr>
<tr>
<td>Bone morphology and osteoporosis</td>
<td>11</td>
</tr>
<tr>
<td>Morphology of bone</td>
<td>11</td>
</tr>
<tr>
<td>Definition of osteoporosis and osteoporotic fractures</td>
<td>14</td>
</tr>
<tr>
<td>Diagnosis of osteoporosis</td>
<td>14</td>
</tr>
<tr>
<td>Aetiology and risk factors</td>
<td>15</td>
</tr>
<tr>
<td>Epidemiology of osteoporosis and fractures in elderly</td>
<td>16</td>
</tr>
<tr>
<td>Caffeine</td>
<td>18</td>
</tr>
<tr>
<td>A brief summary of intake estimates, pharmacology and toxicology</td>
<td>18</td>
</tr>
<tr>
<td>Coffee and coffee consumption</td>
<td>24</td>
</tr>
<tr>
<td>Tea and tea consumption</td>
<td>26</td>
</tr>
<tr>
<td>Coffee/caffeine and tea – association with disease and mortality</td>
<td>27</td>
</tr>
<tr>
<td>Coffee/caffeine</td>
<td>27</td>
</tr>
<tr>
<td>Tea</td>
<td>29</td>
</tr>
<tr>
<td>Coffee, tea, caffeine and osteoporosis</td>
<td>30</td>
</tr>
<tr>
<td>How coffee/caffeine may affect bone – some proposed mechanisms</td>
<td>30</td>
</tr>
<tr>
<td>Tea and bone – some proposed mechanisms</td>
<td>32</td>
</tr>
<tr>
<td>Intake of coffee, tea and caffeine in relation to osteoporotic fractures</td>
<td>33</td>
</tr>
<tr>
<td>Prospective cohort studies</td>
<td>33</td>
</tr>
<tr>
<td>Case-control studies</td>
<td>38</td>
</tr>
<tr>
<td>Cross-sectional study</td>
<td>40</td>
</tr>
<tr>
<td>Conclusions</td>
<td>40</td>
</tr>
<tr>
<td>Intake of coffee, tea and caffeine in relation to bone mineral density</td>
<td>40</td>
</tr>
<tr>
<td>Prospective cohort studies</td>
<td>41</td>
</tr>
<tr>
<td>Case-control studies</td>
<td>43</td>
</tr>
<tr>
<td>Cross-sectional studies</td>
<td>43</td>
</tr>
<tr>
<td>Conclusions</td>
<td>46</td>
</tr>
<tr>
<td>Aims of the studies</td>
<td>48</td>
</tr>
<tr>
<td>General aim</td>
<td>48</td>
</tr>
<tr>
<td>Specific aims</td>
<td>48</td>
</tr>
<tr>
<td>Material and methods</td>
<td>49</td>
</tr>
<tr>
<td>Study populations</td>
<td>49</td>
</tr>
<tr>
<td>The Swedish Mammography Cohort (SMC) (Papers I, III)</td>
<td>49</td>
</tr>
</tbody>
</table>
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>Bone area (per cm²)</td>
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<tr>
<td>BMC</td>
<td>Bone mineral content (g/cm³)</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density (g/cm³)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BUA</td>
<td>Broadband ultrasound attenuation</td>
</tr>
<tr>
<td>bw</td>
<td>Body weight</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Cytochrome P450 1A2</td>
</tr>
<tr>
<td>CV%</td>
<td>Coefficient of variation %</td>
</tr>
<tr>
<td>COSM</td>
<td>Cohort of Swedish Men</td>
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<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
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<td>HR</td>
<td>Hazard Ratio</td>
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<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography-tandem mass spectrometry</td>
</tr>
<tr>
<td>NFA</td>
<td>Swedish National Food Agency</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbons</td>
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<tr>
<td>PIVUS</td>
<td>Prospective Investigation of the Vascularure in Uppsala Seniors</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>SMC</td>
<td>Swedish Mammography Cohort</td>
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<tr>
<td>SMCC</td>
<td>Swedish Mammography Cohort Clinical</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>T-score</td>
<td>The number of SD above or below the mean BMD values for a young healthy adult</td>
</tr>
<tr>
<td>Z-Score</td>
<td>The number of SD above or below the mean BMD values for a population of the same age and sex</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Introduction

Osteoporosis (from the Greek *osteo*, meaning “bone”, *por*, meaning “passageway” and *osis*, meaning “condition”) is a disease in which the density and quality of bone are reduced. These changes, which result in more fragile and porous bones, greatly increase the risk of fracture. This process occurs "silently" and progressively and there are often no symptoms until the first fracture occurs. The most common osteoporotic fractures occur at the hip, spine and wrist. A hip fracture often results in disability and higher mortality; vertebral fractures may have serious consequences, such as loss of height, intense back pain and deformity. In this context it is important to bear in mind that many fractures characterised as osteoporotic are in fact caused by falling, a known strong risk factor for fracture.

In the Western world the incidence of osteoporosis has increased dramatically over the past decades, with the disease now affecting a large proportion of populations worldwide. Furthermore, the highest incidence of osteoporosis that affects postmenopausal women is reported from Scandinavia. The lifetime risk for a middle-aged Swedish woman to be affected by one or more osteoporotic fractures has been estimated to approximately 50% (the corresponding risk for a Swedish man is about 25%).

Osteoporosis is associated with the ageing process, but there are other risk factors that may contribute to the development of the disease: gender, body mass index (BMI), race, genetic disposition, peak bone mass, certain diseases and medications and previous fractures. Further, several lifestyle factors seem important, including smoking, intake of vitamins A and D and calcium, alcohol consumption, low vitamin D status and physical activity.

Some studies indicate that consumption of coffee and total intake of caffeine are associated with increased risk of osteoporotic fractures, whereas others indicate that consumption of tea could have a beneficial effect on bone mineral density (BMD). Results from epidemiological studies regarding these potential associations have not been consistent, however. Nevertheless, to reduce risk of bone loss an official recommendation from the US National Osteoporosis foundation is to avoid more than 3 cups of coffee per day.

This thesis focuses on coffee, tea or caffeine intake and associations with BMD, osteoporosis and fractures typically related to low BMD and osteoporosis. The majority of fractures (except for those of the face) occur in the...
elderly. Previously, classical osteoporotic fractures were restricted to those of the hip, spine, distal forearm and proximal humerus.
Background

Bone morphology and osteoporosis

Morphology of bone

Bone is a specialised supporting tissue characterised by its rigidity and hardness. The major functions of bone are to provide structural support for the body and an environment for bone marrow (both blood forming and fat storage), as well as to protect vital organs and to constitute a mineral reservoir for calcium homeostasis in the body. Bone tissue is composed of inorganic and organic matrices and bone cells (see below). The inorganic part mainly consists of hydroxyapatite (Ca_{10}(PO_{4})_{6}(OH)_{2}) while the main component of the inorganic part is collagen type 1. In addition, the organic part consists of non-collagen proteins (e.g., osteocalcin, osteonectin and proteoglycans).^2^20

There are two types of bone tissue: cortical or compact bone and trabecular synonymous with cancellous or spongy bone (Figure 1). The cortical bone, which constitutes about 80% of the bone mass in adults, forms a shell around parts of the skeleton. The remaining 20% of the bone mass consist of trabecular bone and is mainly located in the vertebrae (spine), the pelvis and the metaphyses of the long bones. It consists of a trabecular network that provides strength by acting as a complex system of internal support. The trabecular bone has more bone cells and the turnover of minerals is more rapid than in the cortical bone.
As shown in Figure 1, the two membranes covering bones are the periosteum, which consists of a dense fibrous membrane on the surface of bones serving as an attachment for tendons and muscles, and the endosteum, which consists of a thin layer of vascular connective tissue lining the marrow cavity. In the periosteum there are nerves and blood vessels nourishing the enclosed bone (not displayed in Figure 1). The organic portion of the matrix is called the osteoid (Figure 2).

The following types of cells compose bone: osteoblasts, osteocytes and osteoclasts. The osteoblasts, which are derived from mesenchymal stem cells, are involved in the production, maintenance and modelling of the osteoid. They are producing factors necessary for regulation of bone formation and resorption: e.g. transforming growth factor beta (TGF-β) and insulin-like growth factor (IGF) \[^{21}\]. The osteocytes are osteoblasts that become incorporated within the newly formed osteoid, which eventually becomes calcified bone. They are involved in the communication between cells in the mineralized bone, which is vital in the adaption of bone tissue to changes in the environment \[^{21}\,^{22}\]. The osteoclasts are large, multinucleated cells located on
bone surfaces derived from haematopoietic stem cells and involved in the remodelling of bone. The process in which bones are shaped or reshaped by independent actions of osteoblasts and osteoclasts is called *modelling* (Figure 2). Modelling of the bone mainly occurs during the time of growing during foetal life, childhood and adolescence and results in gain in skeletal mass and changes in skeletal form. During adult life, bone modelling may continue but not to the same extent as earlier in life.² ²⁰

Old bone tissue in the adult skeleton is constantly replaced by new bone tissue in a process called *remodelling*. This is necessary to maintain bone mass. Modelling and remodelling of bone occur at the same time, beginning in foetal life and continuing to skeletal maturity. Remodelling is then the predominant process during adult life.² ²⁰ It involves complex interactions between osteoclasts and osteoblasts and it is influenced by hormones such as parathyroid hormone (PTH), thyroid-stimulating hormone (TSH)²³, growth hormone, the main active vitamin D metabolite calcitrol as well as cytokines, growth factors, prostaglandins and mechanical stimulation and microdamage²¹ ²⁴. It has been estimated that 5 - 10% of the skeleton in adults is replaced per year by remodelling.² In the trabecular bone the turnover rate is much higher than in the cortical bone because the surface area of trabecular bone is much larger than that of cortical bone.²¹ ²⁵

The process of remodelling, outlined in Figure 3, consists of five phases. It starts with activation (phase 1) that includes stimulation and activation of precursors to osteoclasts. These cells differentiate into mature active osteoclasts by cytokines and growth factors. The process continues with resorption (phase 2), involving the digestion of mineral matrix (old bone) by osteoclasts. Resorption is followed by reversal (phase 3), which is the end of resorption. In the reversal phase the precursors to osteoclasts proliferate and differentiate whereby osteoblasts accumulate in the cavities (*lacunae*) where resorption occurs. After reversal, formation (phase 4) takes place, i.e. the osteoblasts are synthesising a new bone matrix. An often used term for the process when the osteoclasts are followed by osteoblasts is coupling. The last phase is called quiescence (phase 5), where the osteoblasts become resting bone lining cells on the newly formed bone surface.

*Figure 3. Remodelling of bone (with kind permission from the IOF)*
Definition of osteoporosis and osteoporotic fractures

Osteoporosis is a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, resulting in an increase in bone fragility and susceptibility to fracture. Decreased bone strength, which includes both bone quantity and quality, is a prominent feature of osteoporosis. Until recently, osteoporosis was an under-recognised disease, but now perceptions have changed because it has become evident that serious complications may be associated with the disease and that costs to health care and society are high. The typical osteoporotic fractures are those of the hip, spine, forearm and wrist. The incidence of these fractures, especially at the hip and spine, increases with age in both women and men.

There are broadly two kinds of osteoporosis: primary osteoporosis, which is caused by ageing, menopause and lifestyle factors (e.g., food, exercise, smoking and alcohol) and secondary osteoporosis, which is caused by certain diseases and drugs.

To assess risk of fractures and provide adequate treatment (and not least to prevent osteoporosis) it is important to diagnose the disease properly. BMD, assessed by dual energy X-ray absorptiometry (DXA), is used as the gold standard for the diagnosis of osteoporosis. BMD can be expressed as:

1. T-score, which is defined as the number of standard deviations (SD) above or below the mean BMD values for a young healthy adult or
2. Z-score, which is the number of SD above or below the mean BMD values for a population of the same age and sex.

Osteoporosis in women is defined as a BMD value of at least 2.5 SD below the mean value of a young healthy population (T-score ≤ -2.5). The following definitions based on T-scores have been suggested by the WHO.

Table 1. Definitions proposed by the WHO

<table>
<thead>
<tr>
<th>Status</th>
<th>Hip BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>T-score &gt; -1.0 S.D</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>T-score between -1.0 and -2.5 SD</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>T-score &lt; -2.5 SD</td>
</tr>
<tr>
<td>Severe osteoporosis</td>
<td>T-score &lt; -2.5 SD and presence of one or more fragility fractures</td>
</tr>
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</table>

Diagnosis of osteoporosis

Information about present clinical risk factors can provide data for an estimation of the risk of fractures in individual patients. This information, however, can only be used as a screening tool. To confirm the diagnosis, BMD assessment is required in that fractures only occur at advanced stages of the disease. The most well-established method for measuring BMD is DXA.
Generally, diagnosis of osteoporosis is based on assessment of BMD at the spine and proximal femur by DXA.

To detect individuals at high risk who deserve the initiation of treatment, an algorithm providing the absolute 10-year fracture risk in an individual has been developed by the WHO. The algorithm, called the Fracture Index (FRAX® www.shef.ac.uk/FRAX), is based on the individual’s risk factors (e.g., age, sex, weight, height, previous fractures, smoking, consumption of alcohol, rheumatoid arthritis, medications and femoral neck BMD) if they are available. Use of FRAX could be important in countries with limited or no access to densitometry.

Aetiology and risk factors

Osteoporosis is regarded as a multifactorial disease in the sense that there is obviously not one single cause of bone fragility. Genetic and environmental factors influence the development of smaller bones, fewer or thinner trabeculae and thin cortices. During early adulthood, material and strength are maintained by remodelling; by 20 - 30 years of age, peak bone mass is achieved. Depending on skeletal site, up to 50 - 85% of the variance in peak bone mass seems to be genetically determined. With advancing age, less new bone is formed than resorbed in each site remodelled, resulting in bone loss and structural damage. Importantly, the genetic influence on the development of hip fractures and other fractures diminishes with increasing age. The impact of heritability of these fractures after 80 years of age is negligible. Recently, it has been shown that the heritability of BMD decreases with age. These observations indicate that lifestyle factors become even more important at old age in the aetiology of these fractures.

In women, remodelling is even more increased at the time of menopause depending on oestrogen deficiency, whereas bone loss is more continuous in men. At each remodelled site, more bone is then resorbed and less is formed, accelerating bone loss and causing trabecular thinning and disconnection, cortical thinning and porosity. The onset of substantial bone loss begins at about 50 years in women and 65 years in men. However, more women than men are affected by osteoporotic fractures simply because their average lifetime is longer, peak bone mass is lower and loss of trabecular bone proceeds by greater architectural damage, resulting in a skeleton that adapts less effectively to ageing.

There are several potential risk factors for osteoporotic fractures (Table 2), both bone and fall-related. However, these are not equally strong and important at different stages of life. Even though several fractures in elderly are characterised as osteoporotic, only a minority of all women aged 65 years or more in the USA who suffer from these fractures actually have osteoporosis.
High age is thought to be the single strongest risk factor for osteoporotic fractures, but obviously this factor involves several conditions contributing to the effect. There is consensus regarding high age, previous osteoporotic fracture, low BMD (T-score of ≤ -2.5), heredity and systemic treatment with glucocorticoids during at least 3 months being strong risk factors for osteoporotic fractures. In addition, hypogonadism is an important risk factor in both sexes. Less significant risk factors are smoking, alcohol and low calcium intake. To the treatable risk factors belong physical inactivity, impaired balance, low weight/low BMI, cortisone treatment, low bone density, tendency to fall, tobacco smoking, alcohol consumption, low exposure to sunlight and impaired vision. Some of these factors are often associated with advanced age. Regarding secondary osteoporosis, which is more common among younger individuals and men, certain diseases and treatment with some types of drug are risk factors.

Table 2. Summary of proposed risk factors for osteoporotic fractures

<table>
<thead>
<tr>
<th>Female sex</th>
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<tbody>
<tr>
<td>Premature menopause</td>
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<tr>
<td>High age</td>
</tr>
<tr>
<td>Primary or secondary amenorrhoea</td>
</tr>
<tr>
<td>Primary and secondary hypogonadism in men</td>
</tr>
<tr>
<td>Asian or white ethnic origin</td>
</tr>
<tr>
<td>Previous osteoporotic fracture</td>
</tr>
<tr>
<td>Low BMD</td>
</tr>
<tr>
<td>Glucocorticoid therapy</td>
</tr>
<tr>
<td>High bone turnover</td>
</tr>
<tr>
<td>Heredity: family history of hip fracture</td>
</tr>
<tr>
<td>Impaired vision</td>
</tr>
<tr>
<td>Tendency to fall</td>
</tr>
<tr>
<td>Low bodyweight/low BMI</td>
</tr>
<tr>
<td>High body height</td>
</tr>
<tr>
<td>Neuromuscular disorders</td>
</tr>
<tr>
<td>Cigarette smoking</td>
</tr>
<tr>
<td>Excessive alcohol consumption</td>
</tr>
<tr>
<td>Prolonged immobilisation – little or no physical activity</td>
</tr>
<tr>
<td>Low dietary calcium intake</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
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Epidemiology of osteoporosis and fractures in elderly

Osteoporosis is a serious public health concern because of its great prevalence globally. It has been estimated that more than 75 million people in Europe, Japan and the USA were affected by osteoporosis in 2003 and more than 2.3 million fractures were at that time reported from Europe and the USA alone.
Of all postmenopausal women in the USA and Europe, about 30% have osteoporosis, although the proportion is strongly dependent on age. It has been estimated that of these women more than 40% will be affected by one or more osteoporotic fractures during their remaining lifetime. Furthermore, ageing of populations globally will be responsible for a substantial increase in the incidence of osteoporosis in postmenopausal women.

It is well-known that the fracture risk varies considerably between nations and ethnic groups worldwide. The variation between countries in risk of hip fracture and 10-year probability of fractures has been estimated to at least 10-fold. Presently, Denmark, Norway, Sweden and Austria have the highest rates of hip fracture in Europe. Corresponding rates are substantially lower in some other European countries (e.g., Spain, Romania and Croatia). Globally, examples of countries categorised with low risk in this respect are currently China, India, Brazil, Indonesia and the Philippines. Some factors possibly explaining these differences might be heredity, body stature, low level of physical activity, dietary patterns and vitamin D deficiency, although none of these has been shown to be determining factors.

Figure 4. Hip fracture rates for men and women combined in different countries of the world categorised by risk. Where estimates are available, countries are colour coded red (annual incidence > 250/100,000), orange (150–250/100,000) or green (< 150/100,000) From Kanis J. A. et al, 2012. With kind permission from Springer Science and Business Media.

According to The Swedish Council on Health Technology Assessment (SBU), approximately 70,000 fractures per year in Sweden are associated with osteoporosis. Yet, it has been questioned whether all fractures believed to be caused by osteoporosis are actually attributable to osteoporosis.
The lifetime risk for a middle-aged Swedish woman to be affected by one or more osteoporotic fractures has been estimated to approximately 50%, whereas the corresponding risk for a Swedish middle-aged man is about 25% \(^7\). Of the 70,000 fractures per year in Sweden, about 18,000 are hip fractures \(^7\). In recent years the number of hip fractures has increased, mainly because the human life span has increased \(^7\).

Moreover, it should be noted that mortality from hip fractures is high \(^42\). One example may illustrate this point. In a Swedish study of 2,245 incident hip fracture cases and 4,035 randomly selected population-based controls among women 50 - 81 years old, an excess mortality was observed in patients compared with controls \(^43\). The relative risk (RR) of death adjusted for age and previous hospitalisation for serious disease was 2.3 (95% confidence interval [CI] 2.0–2.5) among the patients. The increased risk of death of the hip fracture patients persisted for at least 6 years post-fracture.

The number of worldwide hip fractures in 2000 was estimated to 1.6 million \(^4\) and by 2050 it is estimated that there will be more than 6 million hip fractures, even if age-adjusted incidence rates remain stable \(^44\). In addition to a huge effect on health and social life, osteoporotic fractures have a high economic impact. The global cost for hip fractures is rising and by 2050 it has been estimated to be about 132 billion USD \(^45\). Total fracture cost per year in Sweden has been estimated to 5,639 million SEK, which corresponds to about 3.2% of the total health care costs in the country \(^46\). There are indications that the burden of osteoporosis in the Swedish society is higher than previously presumed, and by 2050, the fracture costs are likely to have increased about five-fold compared with the present cost \(^46\).

Caffeine

A brief summary of intake estimates, pharmacology and toxicology

Caffeine, or 1, 3, 7-trimethylxanthine, is an alkaloid present in more than one hundred plant species. It is believed that caffeine and other methylxanthines have the function of a natural pesticide, contributing to the defence of the plants.

The highest concentrations of caffeine have been detected in the leaves and beans of the coffee plant (*Coffea Arabica* and *Coffea robusta*), in the leaves of the tea plant (*Camelia sininensis*), in the leaves of yerba maté (*Ilex Paraguariensis*), in the berries of guarana (*Paullina cupana*), in the kola nut (*Cola acuminate*) and in the beans from cocoa (*Theobroma cacao*).
Chemically closely related methylxantines are theophylline, which is present in trace amounts in tea, and theobromine, present in cocoa (Figure 5). These are all methylated forms of xanthine (Figure 5).

Figure 5. Chemical structures of caffeine and related compounds

Caffeine intake
There are substantial differences between individuals and cultures regarding the consumption of beverages and foods containing caffeine. Most estimations of caffeine intake have been based on per capita calculations. It is particularly difficult to find national data on total daily intake of caffeine at the individual level. No dietary investigation in Sweden presenting intake data at the individual level has been published in more recent years.

In the USA, the average daily caffeine intake was estimated to be 186 - 227 mg. Corresponding intakes in Canada, Australia, Brazil, Sweden and Denmark have been estimated to 238 mg, 240 mg, 171 mg, 425 mg and 490 mg, respectively. It should, however, be noted that some of these intake estimates date back at least one decade and in some cases almost three decades. The contribution of caffeine derived from coffee seems to be substantial, according to Barone and Roberts, (1996), who estimated that two thirds of the daily intake of caffeine in the USA came from coffee in individuals older than 10 years. Evidently, the intake of caffeine is higher in the Nordic countries than in other parts of the world, primarily because the consumption of coffee in these countries results in a high contribution to the caffeine intake (see “Coffee and coffee consumption” below).

Pharmacokinetics of caffeine
In this brief review focus will be on caffeine, and to some extent, on paraxanthine, which is a primary metabolite of caffeine, although many other compounds in the metabolism of caffeine may be pharmacologically active.
Caffeine is rapidly and almost totally absorbed from the gastrointestinal tract \(^49\). After oral ingestion in humans, peak plasma caffeine concentration is reached after 15 – 120 minutes \(^{50,51}\). After consumption of 3 cups of coffee (corresponding to approximately 300 mg caffeine or 5 mg/kg bw), a peak concentration of caffeine of 10 μg/ml (52 μM) can be observed \(^{52}\). However, plasma concentrations at this level do not last long \(^{52}\).

Caffeine is readily distributed into all tissues of the body, crossing the blood-brain barrier and entering all fluids of the body \(^{49}\). In adult (non-pregnant) humans the half-life of caffeine in plasma is normally in the range of 2.5 - 4.5 hours, although variations up to 9.9 hours have been reported \(^{53,54}\).

Regular caffeine consumption results in a steady state concentration of caffeine and metabolites in the body above concentrations achieved after single doses. Moreover, a dose-dependent metabolism \(^{55-57}\) may contribute to the observed inter- and intra-individual responses to caffeine-containing beverages. There seems to be no major difference in half-life between younger and older male adults \(^{54}\), but lifestyle-related factors like smoking and alcohol consumption may affect half-life as well as hormonal status, diseases and certain drug treatments \(^{58}\). In smokers, a reduction of caffeine half-life by 30 - 50% has been observed in comparison with non-smokers \(^{59-61}\). In contrast, pregnancy \(^{62-64}\) and use of contraceptives \(^{65}\) are known to increase the half-life of caffeine.

The metabolism of caffeine takes place in the liver and the four primary metabolites of caffeine in humans are paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine) and 1,3,7-dimethyluric acid (Figure 6) \(^{66-68}\).

These biotransformation products are further degraded by demethylation, oxidation and ring opening \(^{66-68}\). In the human biotransformation of caffeine the most important step is the 3-methyl demethylation, which results in the formation of paraxanthine. This reaction accounts for about 72 - 90% of caffeine metabolism \(^{50,53,69}\).
Caffeine is extensively metabolised and excreted almost entirely via the kidneys. Of an orally administered dose, less than 2% is excreted unchanged in the urine and at least 98% is transformed in the liver. The most important enzyme in the human metabolism of caffeine is cytochrome P450 1A2 (CYP1A2), which catalyses the demethylation and oxidation of caffeine. CYP1A2 is an important enzyme in the human liver and is involved in the metabolism of a variety of structurally unrelated compounds, including steroids, fatty acids and xenobiotics.

CYP1A2 is known to be inducible and substantial inter-individual variations in the activity of the enzyme, due to genetic and environmental factors, have been observed. Genetically influenced differences in activity and inducibility of CYP1A2 have been reported to affect possible associations between coffee/caffeine intake and the risk of myocardial infarction and hypertension.

In addition to CYP1A2, other cytochrome P450 enzymes (flavin monooxygenase and N-acetyltransferase) have been found to be involved in the metabolism of caffeine. A well-known polymorphism is that in N-acetyltransferase, which results in humans being poor or extensive acetylators of paraxanthine.

Additional to the fact that inter-individual variation of caffeine metabolism depends on genetic factors, the repertoire and amount of caffeine metabolising enzymes may be affected by environmental as well as host factors (e.g., cigarette smoking, certain drugs, diseases and pregnancy).

Molecular mechanisms of caffeine action

Most pharmacological effects of caffeine are explained by competitive antagonism at the level of adenosine receptors, the only known mechanism relevant at the serum levels achieved by intake of caffeine in foods and beverages.

Adenosine occurs naturally in cells and tissue fluids in a nanomolar range under physiological conditions; however, during different forms of distress, concentrations may rise considerably. There are four adenosine receptors (A1, A2A, A2B and A3 receptors) that are located in various organs at different concentrations.

Activation of adenosine receptors by adenosine may result in several physiological effects. Caffeine could be characterised as a non-selective competitive antagonist of adenosine at the A1, A2A and A2B receptors. At low concentration, which is attained after a single cup of coffee (about 4 μM), caffeine is able to significantly inhibit the effects of adenosine on A2A (most potently) and A1 receptors.

In addition, to the effect on the adenosine receptors, caffeine has also been shown to interact with the dopamine system. Caffeine may via these interactions potentiate the neurotransmission of dopamine.
General pharmacological effects of caffeine

Stimulation of the central nervous system is clearly the primary pharmacological effect of caffeine, resulting in such symptoms as increased arousal and vigilance, relief from fatigue and increase in sleep latency, improved performance (decreasing of psychomotor reaction time) and changes in mood.

Furthermore, caffeine affects many other organs and systems in the body. In individuals who have not developed tolerance (see below) a modest increase in blood pressure (both systolic and diastolic) affects heart rate (bradycardia or tachycardia depending on dose). Moreover, neuroendocrine effects (e.g., release of adrenalin, noradrenalin, and renin) have been observed after caffeine intake. However, the final consequences of these effects at the individual level are often difficult to predict since they are complex and sometimes even antagonistic.

In the respiratory system caffeine increases the respiratory rate. The mechanism for this effect is thought to involve sensitising the medullary centre to carbon dioxide.

Caffeine is also known to increase diuresis by increasing the glomerular filtration rate and inhibiting the reabsorption of sodium and water. Renin release from the kidney is increased by caffeine though this effect is transient.

In addition, it has been demonstrated that caffeine stimulates gastric secretion of hydrochloric acid and pepsin, but these effects have also been observed after consumption of decaffeinated coffee. Thus, some components in coffee other than caffeine may be involved in the increase in gastric secretion.

Finally, following caffeine exposure, an increased urinary excretion of calcium has been reported. For more details regarding caffeine intake and relations with calcium balance, see the chapter “Coffee, tea and caffeine and osteoporosis”.

Dependence, tolerance and withdrawal effects

Tolerance to some of the pharmacological effects of caffeine has been reported to develop in humans. For instance, tolerance has been found to develop to cardiovascular effects (effects on blood pressure and heart rate) of caffeine, implying that these effects of caffeine are likely to be transient. In addition, it has been shown that tolerance develops to caffeine effects on diuresis and the levels of adrenalin and noradrenalin as well as to the caffeine effect on renin activity. In these cases tolerance is achieved within a few days. In contrast, tolerance to the effects of caffeine in the central nervous system is equivocal, although adaptive changes occur in the brain.

A caffeine withdrawal syndrome, typically characterized by abstinence symptoms such as headache and fatigue, has been well documented.
Toxic effects and lethal dose

Caffeinism is described as a condition that includes symptoms such as anxiety, tension, headache, insomnia, nervousness, loss of appetite, diarrhoea, dizziness, irritability, decrease in hand steadiness and analgesia. Generally, these symptoms may occur after either a long- or short-term exposure at doses exceeding 7–8 mg/kg bw per day or 500–600 mg/day in adults (corresponding to about 5 cups of coffee), but there are large variations in individual sensitivity towards caffeine.

At higher doses of caffeine, symptoms are aggravating; at a daily dosage of about 20 mg/kg bw, most individuals will experience toxic effects. A variety of toxic effects of caffeine may then appear, mainly related to the CNS, cardiovascular system and gastrointestinal system, in addition to those mentioned above. When toxic effects are experienced, the plasma concentration of caffeine is likely to be higher than 30 μg/ml (150 μmol/l).

The lethal dose of caffeine in man has been estimated to be in the range of 140 - 170 mg/kg bw, equivalent to 8 - 10 grams/day. This dosage would correspond to 60 - 100 cups of coffee.

Coffee and coffee consumption

Though caffeine probably is the most studied component of coffee, coffee is a very complex mixture containing a large number and variety of chemical compounds like carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds. Many bioactive constituents can be found in the unprocessed coffee bean. Processing of the beans (e.g., by roasting) results in a number of additional compounds being formed. An example is the melanoidins, which are high molecular weight nitrogenous and brown-coloured compounds that are likely to exert a number of biological effects.

Among the compounds most often discussed in the context of effects of coffee on human health are caffeine, the diterpenes cafestol and kahweol and phenolic compounds (e.g., chlorogenic acid). Coffee beans may contain up to approximately 1 - 2% of caffeine. The caffeine content in coffee is influenced by the type of coffee beans used, including the processing of the beans, and the method of preparation of the coffee. The average caffeine content of instant coffee, percolated coffee and filter coffee has been estimated to 53, 84 and 103 mg/cup (150 ml), respectively.
Cafestol and kahweol (Figure 7) are found in high amounts in coffee prepared by boiling or pressing (e.g., Scandinavian boiled coffee, Turkish coffee and French press), but in filtered, percolated and instant coffee concentrations are significantly lower. Intake of these diterpenes has been associated with a persistent increase in serum LDL cholesterol. However, the underlying mechanisms for the influence on serum cholesterol have not been clarified.

There are several phenolic compounds in coffee, many of which can be characterised as chlorogenic acids. These are esters formed from quinic acid and trans-cinnamic acid. In coffee the main groups of chlorogenic acid isomers are the caffeoylquinic, feruloylquinic and dicaffeoylquinic acids. In addition, p-coumaroylquinic acids are found in smaller amounts. 5-O-caffeoylquinic acid (Figure 8), often referred simply as to chlorogenic acid, is the most commonly found substance of this type in coffee, accounting for about 50% of the total amount of chlorogenic acids. Like other phenolic compounds, chlorogenic acids are important contributors to the antioxidant properties of coffee. In addition, they contribute to the taste and flavour of this beverage.

Several biological activities, including antioxidant, antimicrobial, anticarcinogenic, anti-inflammatory, antihypertensive and antiglycative properties, have been attributed to melanoidins present in coffee.

Finally, coffee also contains small amounts of substances classified as carcinogens or possible carcinogens in humans. Many of these carcinogens have been formed during the processing of the green coffee bean. Among the most well-known classes of compounds/individual compounds in this respect are polycyclic aromatic hydrocarbons (PAH), heterocyclic amines.
(HCA) and acrylamide, the latter being formed in Maillard reactions during processing.

The contribution of acrylamide from coffee may be substantial. It has been found to range from 33 to 40% of the total intake of this carcinogen from foods in the north of Europe. The possible impact on human health following dietary exposure to acrylamide is still a matter of debate and more investigations to clarify the carcinogenic potential in humans are in progress.

Coffee is globally one of the most popular and consumed beverages with stimulating properties. In 2004, the per capita consumption of roasted coffee in Sweden was estimated to approximately 9 kg. In an international comparison this is a high consumption of coffee. In fact, the Swedish population, like the populations in the other Nordic countries, is reported to have one of the highest consumptions of coffee in the world. According to a recent food survey in Sweden, average coffee consumption was estimated to 337±273 mL per day. Among men, average consumption of coffee was 370 ±290 mL per day and in women 311±256 mL per day. Generally younger persons (18 - 30 years old) had a lower consumption than middle-aged persons (45 -64 years old).

It should, however, be noted that there is considerable variation of volumes of cups in different countries, as well as the types of coffee used. Moreover, doses and preparation methods vary. All these factors are likely to contribute to an uncertainty when comparing consumption patterns between countries.

Tea and tea consumption

Tea is considered an even more chemically complex beverage than coffee. Broadly, there are three types of tea: black, green and oolong. Black tea is completely oxidised, oolong tea is semi-oxidised and green tea is not at all oxidised. Tea contains caffeine and small amounts of theophylline. The caffeine concentration in tea is generally less than 50% of the concentration found in coffee. The average caffeine content of tea has been estimated to 36 - 40 mg per cup of 150 ml. However, variation in caffeine content between different types of tea is considerable.

In addition to the methylxanthines, tea contains a large variety of other types of chemical substance such as flavonoids, polyphenols and tannins. Flavonoids are present both in black and green tea, but the major flavonoids, the catechins (flavan-3-ols), are found in higher quantities in green tea than in black or oolong tea because different methods of processing the tea leaves after harvesting are used. In green tea the major catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) (Figure 9). EGCG is the predominant catechin in green tea, constituting 50 - 75% of the total amount of catechins.
Black tea, on the other hand, contains more oxidised phenolic compounds such as thearubigins and theaflavins. Furthermore, tea is also an important source of fluoride in the diet.

Figure 9. Major catechins in green tea

Except for water, tea is the most consumed non-alcoholic beverage in the world. The most commonly consumed tea in Western countries is black tea, whereas green tea and oolong tea are common in Asian countries. Of the global tea production, 78% is black tea, 20% green tea and 2% oolong tea.

In Europe, Ireland and Great Britain are the dominating markets for tea. Consumption of tea in the Nordic countries, including Sweden, is generally low. In 1997, the per capita consumption of tea in Sweden was estimated to 0.3 kg, which is less than 10% of the Irish per capita consumption.

Coffee/caffeine and tea – association with disease and mortality

Coffee/caffeine

Because of the apparently high correlation between coffee consumption and caffeine intake in most observational studies, it is not easy to separate those two exposures in most epidemiological studies. In fact, associations with caffeine intake per se are seldom investigated in epidemiological studies, but still results are often referred to as associations with caffeine as such. Because the relation between coffee consumption/caffeine intake and dis-
ease/mortality is a huge area to cover, it will only be briefly discussed here with emphasis on coffee.

Previously, coffee drinking was often considered a completely unhealthy habit, but more recent views have changed. It cannot be excluded, however, that some beneficial associations between consumption of coffee and chronic diseases (see below) may be explained by lifestyle factors, associated with intake of this beverage, that have not been adequately controlled for in statistical analyses.

According to Freedman et al, 2012, who examined associations between coffee consumption and total as well as cause-specific mortality in an American cohort of about 400,000 participants, there was an increased risk of death among individuals drinking coffee. However, after adjustment for smoking, which was a more prevalent habit among coffee drinkers, and other potential confounders, an inverse association was noted between consumption of coffee and mortality. With the exception of cancer, inverse associations with coffee drinking were found for deaths attributable to most of the studied diseases in this study (e.g., heart disease, respiratory disease, stroke, injuries and accidents, diabetes and infections).

In addition, the overall results from several epidemiological studies suggest that coffee consumption may be associated with prevention of certain diseases such as type 2 diabetes, Parkinson’s disease, Alzheimer’s disease and liver disease (cirrhosis and hepatocellular carcinoma). Whether consumption of coffee is associated with an increase in cardiovascular disease has been a matter of controversy for a long time. It is well-known that caffeine intake acutely raises blood pressure, especially in hypertensive individuals, but it seems that other components of coffee may be able to counteract these effects to some extent.

Moreover, a great deal of the observed increase in risk of cardiovascular disease has been attributed to the diterpenes cafestol and kahweol in coffee that was not filtered. These compounds, however, have also been associated with anti-carcinogenic activities. When taking lifestyle factors into account, several epidemiological studies indicate that moderate coffee drinking could be associated with beneficial effects regarding cardiovascular health in women. However, there are still unresolved issues with respect to coffee/caffeine and cardiovascular health. Among these are questions on how prolonged coffee consumption would affect blood pressure, how coffee/caffeine intake might affect medical control in hypertensive individuals and how coffee consumption might be associated with risk of stroke. Clearly, more studies are warranted to elucidate these issues.

Generally, in epidemiological studies coffee consumption has not been associated with the development of cancer, though some exceptions may exist for specific cancers in sub-populations. Regarding colorectal cancer, coffee consumption was found to be associated with lower incidence in most case-control studies, whereas no such association was detected in the majori-
ty of prospective cohort studies. However, in a recent large US cohort study consumption of ≥ 4 cups of coffee/day was inversely associated with colon cancer, particularly in the proximal colon. Heavy coffee consumption in men has been associated with an increase in bladder cancer in systematic reviews of case-control and cohort studies, but no dose-response was demonstrated.

Finally, many countries recommend limiting caffeine intake during pregnancy, because of the association observed in some observational studies between high caffeine intake and spontaneous abortion as well as a moderate decrease in birth weight.

Tea

Epidemiological research examining the relation between tea consumption and a variety of chronic diseases is increasing because the phenolic constituents of tea have been found to possess high anti-inflammatory, antioxidant and antimutagenic properties in various biological systems. Since this research field is vast, only a few comments will be made here.

According to the overall scientific documentation, there is growing evidence that tea regularly consumed may decrease the risk of cardiovascular disease, possibly because of the flavonoids present in tea. The association between tea consumption and reduction of the incidence of cardiovascular disease has been demonstrated in cross-sectional and prospective population studies. In addition, animal models have shown that isolated flavonoids occurring in tea can inhibit the development of atherosclerosis.

Results vary concerning the association between tea and cancer, which might be explained by varying contents of the tea catechins in different types of tea. Consequently, the contribution of tea catechins may differ across different populations. Green tea, in comparison with black tea, seems to be more consistently associated with reduced cancer risk, at least for gastrointestinal cancers and lung cancer in non-smokers. The reasons for this may be that there are relatively high concentrations of catechins in green tea compared with black tea or that black tea is less consumed than green tea in the populations studied. There is, however, not yet enough evidence to claim that tea consumption should be recommended in the prevention of cancer.

There is some evidence that the intake of fluoride from tea might protect against caries; however, because studies were few and sample sizes small, more data are needed before an adequate evidence base is available.

Potential harmful associations between tea drinking and health have been considered, e.g. caffeine intake from tea might contribute to dehydration of the body and absorption of non-haem iron from the diet could be negatively influenced by intake of phenolic compounds in tea. None of these potential adverse effects, however, seems to be of importance if tea consumption remains within normal limits (i.e. does not exceed 6 to 8 cups/day).
Coffee, tea, caffeine and osteoporosis

Several dietary factors (e.g., intake of calcium, deficiency of vitamin D and excess intake of vitamin A)\(^{10,116}\) have been proposed to influence the development of osteoporotic fractures. In addition, lifestyle factors (e.g., excessive alcohol consumption and smoking) have been suggested to contribute to fractures. Moreover, high consumption of caffeine-containing beverages has in some studies been found to be associated with increased risk of fracture\(^ {16}\). The mechanisms that might explain this phenomenon, however, are not well elucidated though several hypotheses have been suggested. Finally, it should be noted in this context that there is still considerable controversy whether caffeine is a risk factor for osteoporosis. Nevertheless, avoidance of high coffee consumption is recommended by official organizations such as the US National Osteoporosis Foundation\(^ {18}\).

How coffee/caffeine may affect bone – some proposed mechanisms

Several mechanisms regarding the effects of caffeine on bone have been discussed\(^ {117}\). Generally, there are four principal ways by which an agent would be able to increase skeletal fragility or fracture risk: interference with the bone remodelling process, a decrease in bone mass, an increase in frequency of falls or interference with postural reflexes and a reduction of body fat\(^ {117}\).

Of these options, the first and especially the second seem to be the most studied in relation to caffeine; there are essentially no published data relating caffeine with the third and fourth types of mechanism\(^ {117}\).

A number of studies\(^ {117}\) have investigated the influence of coffee or caffeine on absorption and elimination of calcium and calcium balance in healthy volunteers. In the first study published within this field (Heaney & Recker, 1982)\(^ {118}\), intake of caffeine (consumed as coffee and tea) was significantly associated with a weak negative calcium balance, corresponding to a loss of less than 5 mg calcium per cup of coffee consumed. This effect of caffeine was suggested to be caused by increased excretion of calcium in the urine or to an increased loss of calcium from the intestine.

This work was continued in studies by Massey and co-workers, who demonstrated that a significant acute calcium diuresis was induced by caffeine intake\(^ {119-121}\). According to later investigations\(^ {122}\), however, this effect was found to be biphasic, i.e. after an initial acute rise in calcium levels in the urine, a fall in urinary calcium took place, which resulted in lower amounts of calcium excreted in the urine than previously estimated. Moreover, caffeine may decrease the efficiency of intestinal calcium absorption\(^ {123}\).
Results in subsequent studies have been contradictory, primarily because some of them could not confirm the association between caffeine intake and calcium loss in the urine \(^{124}\) or via faeces \(^{125}\). However, the negative calcium balance persisted in other studies \(^{125}\), where it was concluded that caffeine intake was associated with a small negative calcium loss, corresponding to 4 mg of calcium/cup. Another study in osteoporotic postmenopausal women found the loss of calcium to be 6 mg of calcium/100 ml coffee consumed \(^{118}\). Although the extent of this effect in relation to the development of osteoporosis may be questioned in individuals with a sufficient intake of calcium, it may be more apparent when intake of caffeine is high and ingestion and absorption of calcium is low \(^{126}\) \(^{127}\).

Regarding the first hypothesis i.e. caffeine interfering with bone remodelling process, so far only results from a couple of studies \textit{in vitro} and in experimental animals have been published. According to a study by Tsuang et al, 2006 \(^{128}\) caffeine, in a concentration of 10 mM, has potential deleterious effects on the viability of rat osteoblasts, which may enhance the rate of osteoblast apoptosis. Moreover, Lu et al, 2008 \(^{129}\) reported that cell viability decreased (mainly because of apoptosis) in a dose-dependent manner in human osteoblasts treated with caffeine.

Zhou et al, 2009 \(^{130}\) suggested a new approach. They hypothesised that the real target for caffeine-induced osteoporosis \textit{in vivo} would be bone marrow-derived mesenchymal stem cells (BMSCs), which are precursor cells for osteoblasts. In a subsequent paper Zhou et al, 2010 \(^{131}\) demonstrated that caffeine in high concentrations (0.1 – 1 mM) inhibited the viability and osteogenic differentiation of BMSCs in rats. Another recent study in this area was performed by Su et al, 2013 \(^{132}\) who observed that the effects of caffeine on osteogenic differentiation of primary adipose-derived stem cells and bone marrow stromal cells were biphasic. Caffeine enhanced differentiation to osteoblasts at low concentrations (defined as 0.1 mM) and suppressed it at higher concentrations (defined as 0.3 mM).

Furthermore, Zhou et al, 2009 \(^{130}\) suggested that oestrogen and caffeine can inversely regulate the expression of several genes, which are key factors in bone metabolism. If this presumption proves true, the negative effects of caffeine could be antagonised by oestrogen. In this context, a recent \textit{in vivo} study in rats \(^{133}\) is relevant in that it demonstrated that caffeine and ovariectomy both resulted in deleterious effects on bone metabolism and the combination of both factors in the same group of animals produced an even greater delay in bone repair.

Contrary to several studies suggesting deleterious effects on osteoblasts, Liu et al, (2011) \(^{134}\) found that caffeine (0.005-0.1 mM) enhanced the differentiation of osteoclasts, whereas viability and differentiation of osteoblasts were not affected.

Another mechanism of interest at the cellular level, which has been studied by Rapuri et al, 2001 \(^{135}\) and 2007 \(^{136}\), is related to the observation that
postmenopausal women with a special variant of the vitamin D-receptor (tt) seem to be predisposed to the negative effects of caffeine in terms of a higher rate of bone loss.

It should be noted that in many of the in vitro studies mentioned above, concentrations are considerably higher than peak plasma levels of caffeine reached after human consumption of 2 cups of coffee.

It remains to be demonstrated whether a mechanism that involves direct or indirect effects of caffeine and possibly caffeine metabolites, like paraxanthine, on osteoblasts, osteoclasts or other cells involved in the remodeling process also could be of importance in vivo at dosages of relevance to humans.

Teratogenic effects of caffeine on ossification have been shown in some, but not all, animal studies. The caffeine metabolite paraxanthine has also been found to be teratogenic after administration of very high doses in mice. Primarily, cleft palate and limb malformations were observed. Extremely high doses are required for teratogenic effects of caffeine in experimental animals (rodents). Further, the effects appeared only when the total dose was given on a single occasion by gavage or injection. At present, epidemiological studies do not provide support for an association between caffeine exposure and congenital skeletal malformations in humans.

In conclusion, some studies suggest that a high intake of caffeine in individuals with an insufficient intake of calcium could result in a negative calcium balance and some studies indicate that caffeine could exert direct or indirect deleterious effects on osteoblasts, precursors to osteoblasts or osteoclasts. It is also possible that metabolites of caffeine may be of importance in this context.

Tea and bone – some proposed mechanisms

Tea (especially green tea) is a rich source of flavonoids, predominantly catechins. It seems that bone metabolism (both in vitro and in vivo) is positively affected by these substances. A wide variety of mechanisms have been investigated in vitro and some in vivo (in experimental animals) and some of these might be relevant in this context. Some of the relevant mechanisms include inhibition of bone resorption after addition of PTH in vitro, reduction of osteoclastic cells (but not affecting levels of osteoblasts in vitro), increasing the viability of osteoblastic cells and modulating bone cells in vitro and in vivo.

In addition, flavonoids have been found to improve BMD. Thus, BMD could increase by consuming tea habitually. The effects of polyphenols and tannins in tea may also influence BMD indirectly through elemental mineral metabolism. In addition, bone health can be promoted by the antioxidant activity of tea polyphenols.
Some of the phenolic compounds in tea are weakly oestrogenic, non-steroidal compounds widely occurring in plants. They have been found to stimulate osteogenesis at low concentrations, but inhibit osteogenesis at high concentrations\textsuperscript{143}. One example of this kind of substances occurring in tea is flavonols (such as quercetin)\textsuperscript{143}.

Finally, it has been found that fluoride intake can alleviate osteoporotic progression. It is therefore likely that the relatively high fluoride content of tea leaves may enhance the protective effect on BMD\textsuperscript{17}. The above-mentioned mechanisms may work independently or interdependently\textsuperscript{17}.

Intake of coffee, tea and caffeine in relation to osteoporotic fractures

In the following section the main published studies that have investigated potential associations between consumption of coffee and tea or intake of caffeine and risk of osteoporotic fractures are briefly reviewed. Summarised information about these studies (except for one cross-sectional study), published between 1 January 1988 and 31 December 2012, can be found in Tables 3 and 4. The studies were identified by literature search using the PubMed and the Science Direct databases. Search terms used were: caffeine, coffee, tea, cola, bone health, osteopor*, fracture, epidemiol*, cohort, case-control and cross-sectional study.

Prospective cohort studies

Some population-based prospective cohorts were started in the late 1940s - 1980s, chiefly with the purpose to investigate risk factors for cardiovascular disease, cancer and osteoporotic fractures (primarily hip fractures).

Since the study by Holbrook et al, 1988\textsuperscript{144} is relatively small (details about caffeine intake or statistics were not reported), it will not be discussed here. A part of the Framingham cohort, originally designed to study incidence and prevalence of cardiovascular disease, was investigated by Kiel et al, 1990\textsuperscript{145} to assess intake of caffeine in relation to the risk of hip fracture. A significant increase in the risk of hip fractures (RR = 1.69; 95% CI 1.05 - 2.74) was found after an intake of ≥ 2.5 units of caffeine/day (1 cup of coffee = 1 unit of caffeine; corresponding amount in mg not stated) in comparison with an intake of 0 - 1 unit of caffeine per day. The increased risk was mainly observed in women aged ≥ 65 years. All sources of caffeine were not included and calcium intake was not considered.

In the Nurses’ Health Study\textsuperscript{146}, which is the largest study among the cohorts, reviewed here, with 84,484 participants, a significantly elevated risk of hip fracture (RR = 2.95; 95% CI 1.18 - 7.38), but not with forearm frac-
ture, was observed in women consuming \( \geq 817 \) mg caffeine per day compared with those consuming < 192 mg/day. Moreover, a comparison between participants consuming > 4 cups of coffee/day and those not consuming any coffee resulted in a RR of hip fracture of 3.35 (95% CI 1.32 - 8.49). The number of observed hip fractures was low, which would be expected in a study group consisting of relatively young persons (34 - 59 years). Reported RRs are high in comparison with other studies, but CIs tend to be relatively wide, indicating uncertainty of the estimates.

A borderline negative association (RR = 1.2; 95% CI: 1.0 - 1.5) between intake of caffeine (per 190 mg/day) and the occurrence of hip fracture was found in the Study of Osteoporotic Fractures (SOF) cohort 34. Although the number of participants in this study was large (n = 9,516), the follow-up was rather short (4 years).

No associations between coffee or tea consumption was found in a Japanese cohort study 147 involving 4,573 individuals during 12 – 14 years. Details about measurements of consumption were, however, not reported.

In a large Norwegian cohort study 148, an increased risk of hip fracture was observed at a daily consumption of \( \geq 9 \) cups (1 cup = 115 ml) of coffee (RR = 1.85; 95% CI 1.07 - 3.17). No interaction was found between intake of calcium and coffee. The participants were relatively young and the elevated risk was only found in women.

In contrast, no significant association between caffeine intake and hip fractures could be shown in the Iowa Women's Health Study 149. In this large cohort several sites of fractures, including upper arm, forearm, wrist, hip and spine, were investigated. A statistically significant association between intake of caffeine (dose levels 62.5 - \( \geq 503.8 \) mg/day) and increased risk of wrist fractures was observed. At a dose of \( \geq 503.8 \) mg caffeine/day, the RR was 1.37 (95% CI 1.11 - 1.69). However, RR was essentially the same at all dose levels – i.e. no dose response was observed. In contrast, caffeine intake seemed to be protective regarding the risk of upper arm fractures (RR = 0.67; 95% CI 0.48 - 0.94). In this study, however, case ascertainment might have been a problem since it relied largely on self-reports with no X-ray confirmation of the fractures. Furthermore, it is not clear whether all reported fractures could be classified as osteoporotic. With the use of self-reports of fractures, the problem with survival bias might be introduced since osteoporotic fractures are associated with higher mortality 16.

A considerably smaller study was the investigation carried out by Huopi et al, 2000 150 in Finland. This prospective study comprised 3,068 women 47-56 years old and the follow-up was 3.6 years. No associations between coffee consumption and the risk of osteoporotic fractures of spine, hip, proximal humerus and wrist were found in this study. Relatively few fractures were observed, which might be explained by the short follow-up-time and that the study did not include elderly persons. The mean coffee consumption in the study seems to be similar to the present reported Swedish mean con-
sumption, but no details regarding the measurements of exposure were provided.

An even smaller study concerns the French OFELY cohort. This study included 672 postmenopausal women prospectively followed during 5.3±1.1 years. Coffee and tea consumption as well as intake of caffeine was not significantly associated with a higher risk of fragility fractures. Consumption of these beverages, however, seems to have been very low in comparison with the consumption in Sweden.

Chen et al., 2003 is one of few studies that examined the association between the occurrence of osteoporotic fractures and intake of tea. The number of participants was very large, but follow-up was short. No associations were reported for fractures, but a positive trend of increased BMD with tea drinking was suggested (for details of the study of BMD, see the chapter: Intake of coffee, tea and caffeine in relation to bone mineral density.)

Van Geel et al, 2006 analysed risk factors for clinical fractures in a 5-year prospective study, of 759 postmenopausal women in the Netherlands. The main conclusion was that a recent previous fracture is a relevant factor in detecting postmenopausal women at risk. The HR for coffee intake ≥ 5 consumptions/day was 1.3 (95% CI 0.9 - 1.9), i.e. there was no statistically significant association between consumption of coffee and risk for fractures. The volume of coffee ingested at each occasion of consumption was not defined and more detailed analyses of coffee consumption in relation to fracture risk were lacking.

In a Canadian multicentre study of osteoporosis the relation between fractures and mortality was investigated. During 5 years, 7,753 randomly selected participants (2,187 men and 5,566 women) were followed in this prospective cohort study. Caffeine intake (more than 100 mg/day) was not associated with an increased risk of mortality: on the contrary, a decreased risk was observed (HR = 0.96; 95% CI 0.92 – 0.99).

In a study of risk factors for cervical and trochanteric hip fractures of 1,222 elderly women in Finland, 53 hip fractures were recorded. Of these, 32 were cervical and 21 trochanteric. A statistically significant association (OR = 2.58; CI 95% 1.01 - 6.56) was observed between a current consumption of ≥ 5 cups of coffee/day and the risk of trochanteric fractures. Almost one third of the originally invited women (the frailest group) did not complete the examinations and questionnaire. Potential modifying effects of calcium intake were not taken into account. Furthermore, the volume per cup was not defined.

In Sweden, Trimpou et al, 2010 studied determinants of hip fractures in a cohort of 7,495 men aged 46 - 56 years. During the follow-up of over 30 years, 451 men had a hip fracture. Consumption of coffee (dichotomised as any consumption and none) was associated with lower risk of hip fracture in this cohort. However, according to the authors, this association could be explained by some adverse characteristics among those who did not drink...
coffee (e.g., digestive tract problems). Except for data on consumption of coffee and alcohol, no dietary data were collected in the study.

In an extensive study \(157\) associations between socio-economic factors (and to some extent lifestyle factors) on the risk of hip fractures was investigated in a Dutch cohort (in the final analysis 16,578 individuals were included). The follow-up was nearly 13 years. An inverse association between an income proxy and incidence of hip fractures treated in hospitals was observed, but none between consumption of coffee and hip fracture.

Finally, the study by Määttä et al, 2012 \(158\) is an extension of the study performed by Jokinen et al, 2010 \(155\). The conclusion of this study, which included another 27 to the previously assessed 53 hip fractures, was that there is an association between moderate coffee consumption (defined as more than 3 cups/day) in hypertensive individuals and decreased risk of cervical fractures (HR = 0.4; 95% CI 0.2 - 0.8). In contrast to the results obtained by Jokinen et al, 2010, no association was found between coffee consumption and trochanteric fractures. However, the number of fractures observed in the previous study as well as this study is limited, which could result in considerable changes in risk estimates.

In conclusion, conflicting results have been obtained in cohort studies investigating the association between consumption of coffee, tea or intake of caffeine and osteoporotic fractures. In some cohort studies no association \(144\), \(147\) \(150-153\) \(157\), or only borderline associations \(34\) between intake of coffee/tea/caffeine and hip fractures or any other osteoporotic fractures were observed. In contrast, some studies have found a statistically significant association between high consumption of caffeine/coffee and an increased risk of hip fractures \(145\) \(146\) \(148\) \(155\) and fractures of the wrist \(149\), whereas other studies observed an inverse association between a high consumption of coffee and risk of hip fracture \(156\) \(158\) or mortality \(154\).

However, only one study \(148\) has taken the possible modifying effect of calcium intake into account in the analyses of the risk of fracture associated with a high consumption of caffeine, although this interaction has been suggested in two studies using BMD as outcome \(126\) \(127\). It is possible that the lack of association between intake of coffee/tea/caffeine and hip fractures or any other osteoporotic fractures might be explained by small sample sizes with few fracture cases \(144\) \(147\) \(150\) \(151\) \(153\) \(158\), a short follow-up \(34\) \(150\) \(152\) and a relatively low consumption of coffee \(147\) \(151\). In addition, the fact that consumption of tea and coffee was not considered separately may be relevant \(34\) \(144\) \(154\).
<table>
<thead>
<tr>
<th>First author</th>
<th>Publ. year</th>
<th>n</th>
<th>Age(^a) (years)</th>
<th>Follow-up (years)</th>
<th>Fractures</th>
<th>Exposure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albrand G</td>
<td>2003, 151</td>
<td>672 F</td>
<td>59.1±9.8</td>
<td>5.3</td>
<td>81 all sites(^b)</td>
<td>Coffee, tea, caffeine</td>
<td>NA</td>
</tr>
<tr>
<td>Cummings S</td>
<td>1995, 34</td>
<td>9,516 F</td>
<td>&gt;65</td>
<td>4</td>
<td>192 hip</td>
<td>Caffeine</td>
<td>Borderline increased risk</td>
</tr>
<tr>
<td>Chen Z</td>
<td>2003, 152</td>
<td>91,465 F</td>
<td>50-79</td>
<td>4.1</td>
<td>386 hip 1,809 forearm</td>
<td>Tea</td>
<td>NA</td>
</tr>
<tr>
<td>Fujiwara S</td>
<td>1997, 147</td>
<td>4,573 F</td>
<td>58.5 ±12.2</td>
<td>12-14</td>
<td>55 hip</td>
<td>Coffee, tea</td>
<td>NA</td>
</tr>
<tr>
<td>van Geel A, 2006, 153</td>
<td>759 F</td>
<td>61.0 ±6.8</td>
<td>5-6</td>
<td>95 all sites</td>
<td>Coffee</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Hansen S, 2000, 149</td>
<td>20,035 M 19,752 F</td>
<td>55-69</td>
<td>6.5</td>
<td>1,458 all sites</td>
<td>Coffee, tea, cola, Caffeine</td>
<td>Increased risk Caffeine - wrist Decreased risk Caffeine – upper arm</td>
<td></td>
</tr>
<tr>
<td>Holbrook T, 1988, 144</td>
<td>426 M 531 F</td>
<td>50-79</td>
<td>14</td>
<td>33 hip</td>
<td>Caffeine</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Huopi J, 2000, 150</td>
<td>3,068 F</td>
<td>47-56</td>
<td>3.6</td>
<td>98 all sites</td>
<td>Coffee</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ioannidis G, 2009, 154</td>
<td>2,187 M 5,566 F</td>
<td>66.7 ±9.3</td>
<td>5</td>
<td>859 all sites</td>
<td>Caffeine</td>
<td>Decreased risk</td>
<td></td>
</tr>
<tr>
<td>Jokinen H, 2010, 155</td>
<td>1,222 F</td>
<td>70-73</td>
<td>10</td>
<td>53 hip</td>
<td>Coffee</td>
<td>Increased risk Trochanteric</td>
<td></td>
</tr>
<tr>
<td>Kiel D, 1990, 145</td>
<td>1,353 M 1,817 F</td>
<td>50-84</td>
<td>12</td>
<td>135 hip</td>
<td>Coffee, tea, caffeine</td>
<td>Increased risk Caffeine (F only)</td>
<td></td>
</tr>
<tr>
<td>Lenthe F, 2011, 157</td>
<td>16,578 (M/F?)</td>
<td>25-74</td>
<td>13</td>
<td>192 hip</td>
<td>Coffee</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Meyer H, 1997, 148</td>
<td>20,035 M 19,752 F</td>
<td>47</td>
<td>11.4</td>
<td>213 hip</td>
<td>Coffee</td>
<td>Increased risk (F only)</td>
<td></td>
</tr>
<tr>
<td>Määttä M, 2012, 158</td>
<td>1,222 F</td>
<td>72</td>
<td>13</td>
<td>80 hip</td>
<td>Coffee</td>
<td>Decreased risk Cervical</td>
<td></td>
</tr>
<tr>
<td>Trimpou P, 2010, 156</td>
<td>7,495 M</td>
<td>46-56</td>
<td>30</td>
<td>451 hip</td>
<td>Coffee</td>
<td>Decreased risk</td>
<td></td>
</tr>
</tbody>
</table>

\(^{n}=\)number, \(^{M}=\) male, \(^{F}=\) female, \(^{a}=\) mean age ±SD or age range, \(^{b}=\) all sites” generally include fractures of the hip, spine, wrist or forearm, NA= no association,
Case-control studies

Data on case-control studies are summarised in Table 4. Nieves et al, 1992\textsuperscript{159}, for instance, found an association between an increased risk for hip fractures and a coffee consumption of $\geq 7$ cups/week. In the same study a decreased risk for hip fractures was associated with a tea consumption of $\geq 14$ cups/week. In a Japanese study that included elderly men and women from different areas of Japan\textsuperscript{160} an increased risk of hip fracture was observed after drinking $> 3$ cups of coffee daily. No details from the statistical analyses were reported.

The results regarding tea obtained in the study by Nieves et al, 1992\textsuperscript{159} were confirmed by Johnell et al, 1995\textsuperscript{161} and Kanis et al, 1999\textsuperscript{162} who both observed an association between consumption of tea (all consumption levels) and a decreased risk of hip fractures. In a pilot study\textsuperscript{163}, however, tea drinkers were found to have a higher risk of hip fracture (OR = 22.8; 95% CI 3.86 - 54.23).

In most case-controls studies\textsuperscript{164-168}, however, no statistically significant association between intake of caffeine or coffee and development of fractures was observed. Yet, the chance to find a statistically significant association in the study by Cumming & Klineberg, 1994\textsuperscript{165} may be affected by the relatively large consumption of tea in this study, which investigated the combined effect of coffee and tea as caffeine. A combined exposure of coffee and tea was also studied by Michaëlsson et al, 1995\textsuperscript{166} in a nested case-control design.

Furthermore, the consumption of coffee in some studies\textsuperscript{161,162} that investigated populations in Southern Europe might have been relatively low (according to per capita statistics), although it is only an assumption since details of consumption data were not presented in the articles.

In most of the case-control studies reported in this thesis, data on calcium intake or consumption of dairy products and physical activity were available.

In conclusion, some case-control studies demonstrated that tea consumption was associated with a reduced incidence of hip fractures, whereas an association with coffee might be negative, as shown in two studies.
<table>
<thead>
<tr>
<th>First author</th>
<th>Publ. year</th>
<th>Cases/Control M/F</th>
<th>Age(^a) (years)</th>
<th>Exposure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumming RG</td>
<td>1994, 165</td>
<td>209 hip, 207 controls 105M/311F</td>
<td>65-100</td>
<td>Coffee, tea reported as caffeine</td>
<td>NA</td>
</tr>
<tr>
<td>Jha R</td>
<td>2010, 163</td>
<td>100 (43M, 57F) hip, 100 controls 64.7±13.8</td>
<td>Tea</td>
<td>Increased risk Tea</td>
<td></td>
</tr>
<tr>
<td>Johnell O</td>
<td>1995, 161</td>
<td>2,086 hip, 3,532 controls All F ≥ 50</td>
<td>Coffee, tea</td>
<td>Decreased risk Tea - all consumption levels</td>
<td></td>
</tr>
<tr>
<td>Kanis J</td>
<td>1999, 162</td>
<td>730 hip, 1,132 controls All M</td>
<td>Coffee, tea</td>
<td>NA Coffee Decreased risk Tea - all consumption levels</td>
<td></td>
</tr>
<tr>
<td>Kreiger N</td>
<td>1992, 164</td>
<td>102 hip, 154 wrist 277 controls All F</td>
<td>Coffee, tea</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Michaëlsson K</td>
<td>1995, 166</td>
<td>247 hip, 893 controls All F</td>
<td>Coffee, tea</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ramalho AC</td>
<td>2001, 168</td>
<td>73 hip, 50 control All F</td>
<td>Coffee</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Suzuki T</td>
<td>1997, 160</td>
<td>249 hip cases (43M, 206F) 498 controls (86 M, 412 F) 65-89</td>
<td>Coffee, tea, Japanese green tea</td>
<td>Increased risk Coffee 3 cups/day NA Tea</td>
<td></td>
</tr>
<tr>
<td>Tavani</td>
<td>1995, 167</td>
<td>279 hip, 1061 controls All F</td>
<td>Coffee</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

M=males, F= Females, *mean age ±SD or age range, NA= no association, w= week
Cross-sectional study
One cross-sectional Swedish study will be briefly mentioned. This study included 619 men and women (mean age 70 years) and used both prevalent fractures and BMD as outcomes. Coffee (number of cups/day) was the only exposure variable. Mean daily intake of coffee was 3.2 cups.

In this study no statistically significant association was observed between consumption of coffee and previous fractures or between consumption of coffee and bone mineral content (BMC) of the calcaneum.

Conclusions
Potential associations between intake of coffee or caffeine and osteoporotic fractures have been investigated in a rather limited number of prospective cohort studies and case-control studies. Some of the largest prospective cohort studies have observed an association or borderline association between high intake of coffee/caffeine and osteoporotic fractures, whereas others have not demonstrated such associations. A possible positive association between tea consumption and hip fractures was demonstrated in some case-control studies, i.e. a reduction of the relative risk of hip fractures was observed.

The results from these studies should be interpreted cautiously in that the possible modifying effect of calcium intake has seldom been taken into account in the analyses. Moreover, many of the studies were small with few fracture cases. In addition, the consumption of coffee was low in some studies. It may also be important that consumption of tea and coffee was not considered separately in some studies, since there are indications that consumption of tea could have a positive influence on BMD, which could counteract potential negative influence of coffee.

Intake of coffee, tea and caffeine in relation to bone mineral density
BMD is widely used as a proxy variable for osteoporotic fracture in epidemiological studies of osteoporosis. Estimating the fracture risk from measurements of BMD is analogous to estimating the risk of stroke from blood pressure measurements. It should, however, be recognised that a normal BMD does not mean that fractures will not occur; rather, it means that there is less risk.

Considerably more observational studies on the intake of caffeine/coffee/tea and associations with BMD have been published than studies on the intake of caffeine/coffee/tea and occurrence of osteoporotic fractures. The majority of the studies in this field have been performed with a
cross-sectional design, although some longitudinal studies have been performed. Case-control studies are scarce.

In the following, the main studies published between 1 January 1988 and 31 December 2012, are summarised. Information about the prospective cohort studies and the cross-sectional studies is also given in Tables 5 and 6. The studies were identified by literature search using the PubMed and the Science Direct databases. Search terms used were: caffeine, coffee, tea, cola, osteopor*, bone mineral density/content, epidemiol*, cohort, case-control and cross-sectional study.

Prospective cohort studies

The largest studies investigating BMD included nearly one thousand to five thousand participants. However, most studies are relatively small, (less than one hundred to several hundred participants). The cohort studies of BMD are summarised in Table 5.

Most studies included predominantly females and have been published in the last two decades. In most of these studies caffeine was the exposure variable and all reported sources of caffeine were pooled into a total estimate of caffeine exposure. A significant decrease in BMD in relation to caffeine intake was noted by Harris and Dawson-Hughes, 1994, in women with a low intake of calcium. Generally, no association between coffee consumption and BMD was observed in the prospective cohort studies. However, Korpelainen et al, 2003 observed a decrease in BMD of the radius and in BUA of the calcaneus in lean women consuming ≥5 cups of coffee daily. In contrast, Videman et al, 2007 demonstrated that coffee consumption was associated with an increase in femoral BMD in a study of 70 monozygotic twins.

In three studies tea was the exposure variable. The results of the studies of tea consumption indicate that there is a positive association between tea and BMD, i.e. tea consumption is associated with an increase in BMD. The results of and suggest that the level/duration of tea consumption may be of importance for the magnitude of increase in BMD.

As in the cohort studies of fractures (Table 3), absence of associations may be due to small sample sizes and a relatively low consumption of coffee or low intake of caffeine. Another explanation may be that the consumption of tea and coffee was not considered separately.
<table>
<thead>
<tr>
<th>First author</th>
<th>Publ. year</th>
<th>n</th>
<th>Age(^a) (years)</th>
<th>Follow-up (years)</th>
<th>Exposure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen Z</td>
<td>2003, 152</td>
<td>4,979 F (subgroup in cohort)</td>
<td>50-79</td>
<td>7</td>
<td>Tea</td>
<td>Total body: positive trend Hip, spine: NA</td>
</tr>
<tr>
<td>Devine A</td>
<td>2007, 172</td>
<td>164 F</td>
<td>74.8 ± 2.8</td>
<td>4</td>
<td>Tea</td>
<td>Hip: BMD in tea drinkers greater than in non-tea drinkers</td>
</tr>
<tr>
<td>Hannan M</td>
<td>2000, 177</td>
<td>304M 596F</td>
<td>74.5 ± 4.5</td>
<td>4</td>
<td>Coffee, tea, caffeine</td>
<td>Hip, radius, spine: NA</td>
</tr>
<tr>
<td>Harris S</td>
<td>1994, 127</td>
<td>205 F</td>
<td>61 ± 5</td>
<td>1</td>
<td>Coffee, tea, cola, chocolate, caffeine</td>
<td>Spine, total body: Decrease - at low calcium intake</td>
</tr>
<tr>
<td>Holm K</td>
<td>2002, 178</td>
<td>368 F</td>
<td>35-60</td>
<td>2</td>
<td>Coffee, tea, cola, caffeine</td>
<td>Spine: NA</td>
</tr>
<tr>
<td>Korpelainen R</td>
<td>2003, 170</td>
<td>1,222 F</td>
<td>70-73</td>
<td>?</td>
<td>Coffee</td>
<td>BMD Radius: Decrease in lean women Ultrasound (BUA) Calcaneus: Decrease in lean women</td>
</tr>
<tr>
<td>Lloyd T</td>
<td>1998, 173</td>
<td>81 F</td>
<td>12-18</td>
<td>6</td>
<td>Coffee, tea, cola, chocolate, caffeine</td>
<td>Total body, hip: NA</td>
</tr>
<tr>
<td>Lloyd T</td>
<td>2000, 174</td>
<td>92 F</td>
<td>63.3 ± 4.4</td>
<td>2</td>
<td>Coffee, tea, cola, chocolate, caffeine</td>
<td>Total body, hip: NA</td>
</tr>
<tr>
<td>Packard PT,</td>
<td>1996, 175</td>
<td>145 F</td>
<td>20-30</td>
<td>1.6-4</td>
<td>Caffeine</td>
<td>Total body, spine: NA</td>
</tr>
<tr>
<td>Reid I</td>
<td>1994, 176</td>
<td>122 F</td>
<td>58.1 ± 5.0</td>
<td>2</td>
<td>Caffeine</td>
<td>Total body, spine, femur: NA</td>
</tr>
<tr>
<td>Wu C H</td>
<td>2002, 17</td>
<td>497 M</td>
<td>&gt;30</td>
<td>1, 5, 6-10</td>
<td>Tea</td>
<td>Hip, spine, total body: Increase after consumption during ≥10 years</td>
</tr>
</tbody>
</table>

\(n=\) number, M= male, F= female, \(^a\) mean age ±SD or age range, NA= no association
Case-control studies

No statistical significant difference in consumption of caffeine was found between men with osteoporosis and controls \(^{179}\). High tea consumption (4 - 7 cups/day) was found to be a protective factor for the risk of osteoporosis in a multicentre study of Iranian and Indian women \(^{180}\). The study comprised 363 subjects (178 osteoporotic and 185 with normal BMD) from Iran and 354 subjects (203 osteoporotic and 151 with normal BMD) from India.

Cross-sectional studies

The cross-sectional design is the most frequently used in studies (displayed in Table 6) of bone density and intake of coffee/tea/caffeine. Few cross-sectional studies have included males and in most studies the studied populations consisted of females only.

A negative association between coffee (or caffeine) and BMD was found in the studies by Cooper et al, 1992 \(^{181}\), Hernandez-Avila et al, 1993 \(^{182}\), Barrett-Connor et al, 1994 \(^{126}\), Rubin et al, 1999 \(^{183}\), Ilich et al, 2002 \(^{184}\), Bauer et al, 2003 \(^{185}\), and Barbour et al, 2010 \(^{186}\). In addition, a negative association between caffeine and BMC was in the study by Wetmore et al, 2008 \(^{187}\), but only in participants using oral contraceptives. In yet another study \(^{188}\) a high consumption of coffee (defined as > 3 cups/day) was found to be associated with a significant increase of osteoporosis of the spine, whereas osteoporosis at any site was significantly decreased. One limitation of this study concerns selection bias in that the population was recruited among hospital staff and university students only. A borderline significant negative association was noted in Hoch et al, 2009 \(^{189}\). In contrast, BMD was significantly increased in relation to consumption of tea in the studies by Hoover et al, 1996 \(^{190}\), Hegarty et al, 2000 \(^{191}\), Devine et al, 2007 \(^{172}\) and Muraki et al, 2007 \(^{192}\). In the study by Hamdi Kara et al, 2007 \(^{193}\), T-scores of participants consuming > 2 cups of tea daily tended to be higher than of those consuming < 2 cups, but the difference was not statistically significant.

In the Rancho Bernado Study by Barrett-Connor et al, 1994 \(^{126}\) and in the study by Ilich et al, 2002 \(^{184}\) an association between coffee or caffeine intake and a reduction in BMD was observed but only when calcium intake levels were low. In the study \(^{126}\) this intake corresponded to < 1 glass of milk daily, (i.e. approximately 230 mg calcium according to the food database of the National Food Agency \(^{194}\)), whereas in the study \(^{184}\) it corresponded to an intake of less than 750 mg calcium/day. It should be noted that several studies were small (see Table 6) and the consumption of coffee or intake of caffeine was low or modest in other studies \(^{181} 195\ 196\). Furthermore, no separate analyses of coffee and tea were carried out in many of the studies \(^{181} 183\-185\ 195\-204\). This may not be optimal because both beverages contain several other bioactive substances that may modify the effects of caffeine.
As in the cohort studies, there are some indications that tea consumption could be associated with an increase in BMD, whereas coffee/caffeine consumption in some studies was associated with a decrease in BMD. In two studies the latter association was only observed when calcium intake was low. As always, the results from such studies must be interpreted cautiously, especially regarding causality.

Table 6. Cross-sectional studies – Bone mineral density

<table>
<thead>
<tr>
<th>First author</th>
<th>Publ. year</th>
<th>n</th>
<th>Age (years)</th>
<th>Exposure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atalar E</td>
<td>2009, 204</td>
<td>131 M</td>
<td>20-75</td>
<td>Caffeine (coffee, tea, cola, chocolate)</td>
<td>Spine, hip: NA</td>
</tr>
<tr>
<td>Barett-Connor E</td>
<td>1994, 126</td>
<td>980 F</td>
<td>50-98</td>
<td>Coffee</td>
<td>Spine, hip: Decrease in participants not drinking milk daily</td>
</tr>
<tr>
<td>Barbour K,</td>
<td>2010, 186</td>
<td>1,172 M</td>
<td>69-97</td>
<td>Caffeine</td>
<td>Hip: Decrease</td>
</tr>
<tr>
<td>Bauer DC</td>
<td>2003, 185</td>
<td>9,704 F</td>
<td>≥ 65</td>
<td>Coffee, tea, cola, caffeine</td>
<td>Distal radius Weak negative association</td>
</tr>
<tr>
<td>Cauley J</td>
<td>2005, 203</td>
<td>5,995 M</td>
<td>≥ 65</td>
<td>Caffeine</td>
<td>Hip, spine: NA</td>
</tr>
<tr>
<td>Cooper C</td>
<td>1992, 181</td>
<td>298 F</td>
<td>Random sample of adults</td>
<td>Coffee, tea, cola, caffeine</td>
<td>Femoral shaft: Caffeine: Decrease</td>
</tr>
<tr>
<td>Demirbag D</td>
<td>2006, 205</td>
<td>200 F</td>
<td>58.9 ± 8.5</td>
<td>Coffee</td>
<td>Spine, hip: NA</td>
</tr>
<tr>
<td>Devine A</td>
<td>2007, 172</td>
<td>1,207</td>
<td>79.8 ± 2.7</td>
<td>Tea, coffee</td>
<td>Hip: Increase in tea drinkers than in non-tea drinkers</td>
</tr>
<tr>
<td>El Maghraoui A</td>
<td>2010, 188</td>
<td>592 M</td>
<td>20-79</td>
<td>Coffee</td>
<td>Spine: High consumption associated with spine osteoporosis</td>
</tr>
<tr>
<td>First author</td>
<td>Publ. year Reference</td>
<td>n</td>
<td>Age (years)</td>
<td>Exposure</td>
<td>Main results Site of BMD Measurement</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------</td>
<td>-------</td>
<td>-------------</td>
<td>----------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Forsmo S 2001, 207</td>
<td></td>
<td>1,652</td>
<td>50-59</td>
<td>Coffee</td>
<td>Distal, ultradistal radius; NA</td>
</tr>
<tr>
<td>Glynn N 1995, 199</td>
<td></td>
<td>523 M</td>
<td>66.6</td>
<td>Coffee, tea, cola, caffeine</td>
<td>Hip: NA</td>
</tr>
<tr>
<td>Hamdi Kara 2007, 193</td>
<td></td>
<td>724F</td>
<td>57.6 ± 9.6</td>
<td>Coffee, tea</td>
<td>T-score: NA</td>
</tr>
<tr>
<td>Hegarty VM 2000, 191</td>
<td></td>
<td>1,256 F</td>
<td>65-76</td>
<td>Tea only</td>
<td>Several sites: Increase in tea drinkers compared to non-tea drinkers</td>
</tr>
<tr>
<td>Hoch A 2009, 189</td>
<td></td>
<td>160 F</td>
<td>13-18</td>
<td>Caffeine</td>
<td>Spine, hip, total body: Borderline significant association with low BMD</td>
</tr>
<tr>
<td>Hoover PA 1996, 190</td>
<td></td>
<td>62 F</td>
<td>62.9 ± 6</td>
<td>Tea</td>
<td>Femur: Increase</td>
</tr>
<tr>
<td>Hernandez-Avila M 1993, 182</td>
<td></td>
<td>281 F</td>
<td>50-60</td>
<td>Coffee, tea, cola, caffeine</td>
<td>Radius: Decreased BMD in relation to all exposure</td>
</tr>
<tr>
<td>Ilich J 2002, 184</td>
<td></td>
<td>136 F</td>
<td>68.6± 7.1</td>
<td>Caffeine</td>
<td>Most skeletal sites: Decrease Attenuated at Ca intake ≥750 mg/day</td>
</tr>
<tr>
<td>Ilich J 2009, 208</td>
<td></td>
<td>120 F</td>
<td>43-79</td>
<td>Coffee, tea</td>
<td>Spine, hip: NA</td>
</tr>
<tr>
<td>Johansson C 1992, 169</td>
<td></td>
<td>619 (M+F)</td>
<td>70</td>
<td>Coffee</td>
<td>BMC calcaneum: NA</td>
</tr>
<tr>
<td>Krahe C 1997, 200</td>
<td></td>
<td>60 F</td>
<td>40-50</td>
<td>Coffee, caffeine</td>
<td>Spine, hip: Increased BMD at higher caffeine intake (due to confounding)</td>
</tr>
</tbody>
</table>
Table 6. Cross-sectional studies – Bone mineral density cont.

<table>
<thead>
<tr>
<th>First author</th>
<th>Publ. year Reference</th>
<th>n</th>
<th>Age (years)</th>
<th>Exposure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyriazopoulos P</td>
<td>2006, 209</td>
<td>300 M</td>
<td>18-30</td>
<td>Coffee, tea</td>
<td>Radius: NA</td>
</tr>
<tr>
<td>Lloyd T</td>
<td>1997, 201</td>
<td>138 F</td>
<td>55-70</td>
<td>Coffee, caffeine</td>
<td>Total body, hip: NA</td>
</tr>
<tr>
<td>Reyes M</td>
<td>2004, 210</td>
<td>52 M, 42 F</td>
<td>42</td>
<td>Coffee, tea</td>
<td>Spine, hip: NA</td>
</tr>
<tr>
<td>Rico H</td>
<td>2002, 196</td>
<td>93 F</td>
<td>57.3 ± 7.1</td>
<td>Caffeine</td>
<td>Ultrasound non-dominant hand: NA</td>
</tr>
<tr>
<td>Ruffing JA</td>
<td>2006, 211</td>
<td>755 M</td>
<td>Mean 18.7</td>
<td>Caffeinated drinks</td>
<td>Calcaneus (all) and several sites (subgroup): NA</td>
</tr>
<tr>
<td>Saitoglu M</td>
<td>2007, 212</td>
<td>70 M</td>
<td>45-65</td>
<td>Coffee, tea</td>
<td>Spine, hip: NA</td>
</tr>
<tr>
<td>Travers-Gustafson D</td>
<td>1995, 213</td>
<td>899 F</td>
<td>F: 65.6</td>
<td>Coffee used as a proxy for caffeine</td>
<td>Ultrasound patella: NA</td>
</tr>
<tr>
<td>Wetmore CM</td>
<td>2008, 187</td>
<td>324 F</td>
<td>≥ 65</td>
<td>Tea</td>
<td>Spine, hip, total body: Decreased BMC in spine - but only in participants using oral contraceptives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>625 F</td>
<td>14-40</td>
<td>Coffee, tea, cola</td>
<td></td>
</tr>
</tbody>
</table>

n= number, M= male, F= female, *mean age±SD or age range, NA= no association

Conclusions

Most prospective cohort studies have not observed statistically significant associations between intake of coffee/tea/caffeine and BMD of the
hip/spine/total body. In a couple of the cohort studies and in one case-control study, however, a positive association between tea and BMD was indicated (i.e. an increase in BMD was observed).

Similar results were found in the cross-sectional studies, which constitute the vast majority of the studies in this field. It should be kept in mind that in all epidemiological analyses (especially in cross-sectional studies) causal inference is difficult.
Aims of the studies

General aim
The overall aim of this thesis was to investigate whether long-term consumption of coffee, tea or intake of caffeine is associated with adverse effects on bone health in men and women.

Specific aims
The specific aims were to:

Study the association between consumption of coffee, tea and caffeine and the risk of incident fractures in middle-aged and elderly women using a prospective cohort design (Papers I, III).

Explore the risk of fractures in relation to coffee consumption in middle-aged and elderly men in a prospective cohort study (Paper IV).

Investigate the relation between consumption of coffee and BMD of the proximal femur in elderly men and women, taking the genotype of CYP1A2 into consideration (Paper II).

Study BMD, incidence of osteoporosis and rate of low-energy falls in relation to coffee consumption in middle-aged and elderly women (Paper III).

Consider potential interactions with calcium intake in all studies (Papers I – IV).
Material and methods

Study populations

The Swedish Mammography Cohort (SMC) (Papers I, III)
The Swedish Mammography Cohort (SMC) was established in 1987 - 1990. All 90,303 women residing in two Swedish counties (Uppsala and Västmanland) and born between 1914 and 1948 received a mailed invitation to a mammography screening. Enclosed with this invitation was a food frequency questionnaire (FFQ) covering diet and lifestyle, which was completed by 66,631 (74%) of the women. After exclusions, 61,433 women in the cohort were eligible for analysis. (A flow chart of the SMC is shown in Figure 10).

To all those who were still living in the study area a second, expanded questionnaire, was sent in 1997. In the expanded FFQ, questions regarding smoking, use of HRT and physical activity were also included. The response rate of this follow-up questionnaire was 70%.

In study I, the participants of the SMC, from Uppsala County only, were investigated for consumption of coffee, tea and intake of caffeine and any potential associations between the incidences of osteoporotic fractures. The reason for not including participants from Västmanland County was that information about fractures from this county was not complete at the time of the study.

Of the 48,517 invited women in Uppsala, 34,916 (72%) participated at the baseline investigation. After exclusions according to predefined criteria, the final sample comprised 31,527 women. The predefined criteria for exclusions in the SMC were: missing or incorrect ID numbers, not falling within the age range 40 - 76 years, missing the return date on the questionnaire, moved out of the study area or missing date of death (for participants who died during follow-up), extreme energy intake, extreme unreasonable values for height and weight and prevalent cancers at study entry. In study I, the end of the follow-up was 31 December 2000.

In study III, the full SMC was investigated. In this study fracture data for 61,433 participants at baseline and 38,984 participants at follow-up in 1997 were available for analysis. In study III, the end of the follow-up was 31 December 2008.
The Swedish Mammography Cohort Clinical (SMCC) (Paper III)

A randomly selected sub-cohort of the SMC in the city of Uppsala was invited to a third dietary investigation and bone scans. Between November 2003 and October 2009 this sub-cohort was investigated by DXA (DXA, Lunar Prodigy, Lunar corp., Madison, WI, USA). In addition, the participants provided blood and urine samples, had height and weight measurements taken and completed a third questionnaire (similar to the 1997 FFQ) on diet and lifestyle factors before the clinical examination.

The participation rate was 65% and the sub-sample consisted of 5,022 women. BMD (g/cm²) was determined at the hip, lumbar spine (L1 - L4) and total body. In study III, consumption of coffee, in relation to BMD and the occurrence of osteoporosis, was investigated in the SMCC.

Prospective Investigation in Uppsala seniors (PIVUS) (Paper II)

Eligible for the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) were all 70-year-old individuals living in Uppsala, Sweden in 2001 – 2004. Within 2 months of their 70th birthday, 2,025 of these individuals (49% men and 51% women) were randomly selected and invited to participate in the study. Eventually, 1,016 (50%) of the invited persons participated in the PIVUS study.

The aim of the PIVUS study was to evaluate different measurements of endothelial function and arterial compliance to predict cardiovascular dis-
ease. Examinations included measurements of blood pressure and anthropology, blood sampling after an overnight fast, routine medical history and assessment of BMD using DXA. In addition, 7-day food consumption recordings were performed. Furthermore, medical/drug history and information about lifestyle factors were collected.

In study II, dietary data and data from BMD measurements were available for analysis for 717 individuals (359 men and 358 women) in PIVUS.

**Cohort of Swedish Men (COSM) (Paper IV)**

The Cohort of Swedish Men (COSM) was formed in the autumn of 1997. All men born between 1918 and 1952 (45 - 79 years of age) and residing in Örebro and Västmanland Counties, central Sweden, received written information about the study and a self-administered questionnaire that included nearly 350 items on diet and other lifestyle factors.

Of those invited, 48,645 returned a completed questionnaire (response rate 49%). Predefined exclusion criteria were: erroneous or a missing national identification number, implausible values for total energy intake (i.e. 3 SDs from the log-transformed mean energy intake), a cancer diagnosis (except for non-melanoma skin cancer and before 1 January 1998) and men who had passed away before 1 January 1998. Furthermore, participants with lacking data on coffee consumption (2,361 individuals) were excluded. After these exclusions, 42,978 men remained for analysis. (A flow chart of COSM is displayed in Figure 11.)

This large population-based cohort is representative of Swedish males aged 45 - 79 years in terms of age distribution, educational level and prevalence of overweight. The questionnaire was identical to that used in the SMC in the 1997 investigation. Questionnaire data on height (at age 20), weight, education, civil status, employment, alcohol consumption, smoking habits, cortisone use and physical activity were available. Occupational physical activity, walking/cycling duration per day and exercise duration per week have been reported for the past year according to five predefined categories. Data on smoking included smoking status (never, past, current), duration and the number of cigarettes smoked, on average, at various ages.
Dietary assessments including assessments of coffee/tea consumption

Paper I

In the FFQ questions on 67 foods items commonly consumed in Sweden were included. The women were asked to indicate how often on average per day during the past 6 months they had consumed these food items. There were also questions about the consumption of regular coffee and black tea. The main caffeine sources assessed were coffee, tea, caffeinated soft drinks and chocolate. The categories of consumption ranged from never/seldom to ≥ 4 times daily.

Age-specific portion sizes were used in order to calculate nutrients. They were based on mean values of 5,922 days of weighed food records from 213 women participating in the validation of the FFQ, in order to. By multiplying the frequency of consumption of each unit of food by the nutrient content of specified portions derived from a database created by the Swedish National Agency (NFA) \(^{215}\), intake of caffeine, alcohol and nutrients (such as calcium, phosphorous, vitamin D and A) and energy intake were computed. One cup (150 ml) of coffee was assumed to contain 80 mg of caffeine according to information from the Institute of Environmental Medicine, Karolinska Insti-
The validity of the nutrient estimates based on self-reported food frequencies was evaluated four times 3 - 4 months apart in a sub-sample of 129 participants from the cohort during 7 days. Spearman’s correlation coefficients between the FFQ and the food records were 0.6 for coffee and 0.8 for tea (A. Wolk, unpublished data 1992).

Paper II
Dietary habits were registered in 84% of the participants (i.e. 850 individuals). Each participant used a pre-coded food diary and recorded his or her food consumption during 7 consecutive days. This pre-coded food diary had been prepared and previously used by the NFA and Statistics Sweden in a food survey of 3,000 households in 1989. The questionnaire has been validated. Consumption of coffee and tea was registered six times daily (breakfast, lunch, supper, between meals and in the evening).

By using a computerised program and information about energy and the nutrient contents of foods from the NFA, the daily intake of energy, caffeine, alcohol and selected nutrients, including calcium, vitamin D and A, was calculated. One cup of filtered coffee (150 mL) was estimated to contain approximately 100 mg caffeine and one cup of tea (200 mL) about 50 mg of caffeine. The caffeine content of the consumed coffee and tea was not analysed.

Paper III
In study III, data from three self-administered FFQs were used. The first FFQ from 1987 has been described above (Study I). Dietary intake in 1997 was assessed at baseline using a 96-item FFQ (second FFQ). Frequency of consumption was reported according to eight predefined categories ranging from never/seldom to ≥ 3 times/day. In the third FFQ (2008-2009) a similar questionnaire was used. As in study I, intake of nutrients was estimated by multiplying the nutrient content of age-specific portion sizes by the consumption frequency of each food item. By using the residual method, intake of nutrients was adjusted for total energy intake (set at 1700 kcal, which is the average intake in the study population).

In all three FFQs consumption of coffee was recorded. In the FFQ from 1987 (baseline), the participants were asked how often, on average, during the previous 6 months they had consumed coffee, black tea and other foods and beverages according to eight predefined categories. In the FFQs from 1997 (second) and 2008 - 2009 (third) the participants were asked open-ended questions on the number of cups of coffee they had been drinking per day or week during the previous year.

Coffee consumption was assessed according to the following categories: < 1, 1, 2-3 and ≥ 4 cups daily. Coffee and tea are almost exclusively con-
sumed in their regular forms in Sweden since the consumption of decaffeinated coffee and tea is very low.

Paper IV
The questionnaire in study IV was identical to that used in the SMC in the 1997 investigation. Dietary intakes were assessed in the same way as in SMC 1997.

A validation study of the FFQ used in COSM in 1997, has been performed. This study focused on validity of nutrient estimates. As reference method, fourteen 24-h recall telephone interviews during 1 year were used. A sample of 248 men, 40 - 74 years old, from the area of the study was interviewed. Mean Spearman’s correlation coefficients were for macronutrients: 0.65 and for micronutrients: 0.62.

Outcomes

Bone measurements (Papers I – IV)
Incident fractures and BMD were the outcomes used to study the association between intake of coffee, tea and caffeine and bone health. In addition, the incidence of osteoporosis, according to the definition established by the WHO (1994) was determined in study III.

BMD (Papers II and III)
In papers II and III, all BMD measurements (g/cm²) were performed using DXA (DPX, Lunar Prodigy, Lunar corp., Madison, WI, USA).

In paper II, 898 of the 1,016 members of the PIVUS cohort underwent BMD measurements for total proximal femur, femoral neck and trochanteric regions of the proximal femur 2 years (on average) after the baseline investigation. When applicable, both extremities were used in the calculation. The precision error of the DXA measurements of the total proximal femur was calculated to be about 0.7%.

In paper III, BMD measurements were done at three sites (total body, proximal femur [total hip] and L1 - L4 of the lumbar spine) in the sub-cohort SMCC. Based on triple measurements in 15 subjects, the precision error for BMD was 0.8 - 1.5%, depending on the site. The long-term precision coefficient of variation (CV) was less than 1%.

Osteoporosis (Paper III)
The incidence of osteoporosis was calculated in the investigation of the SMCC in paper III. Osteoporosis was defined as a BMD ≤ 2.5 SD below the
mean of a young adult reference range at the total hip, the femoral neck or the spine \textsuperscript{26,220}.

**Fractures (Papers I, III and IV)**

In paper I, typical osteoporotic fractures of all types were identified in women in Uppsala County. These include all fractures of the proximal femur, i.e. hip fractures, fractures of the pelvis, spine, distal forearm and proximal humerus. The identification of the fracture cases in the cohort was performed by matching the unique personal identification number of the study participants with the local out-patient registers, hospital discharge records and X-ray records from January 1988 through December 2000. Ascertainment of hip fracture cases was completed by use of the Swedish inpatient register, a register covering the whole country.

Although most fractures in the elderly are associated with low BMD, only a minority of those who suffer these fractures have osteoporosis according to the WHO definition\textsuperscript{19}. We therefore used another designation than osteoporotic fracture to denote the composite end-point of fractures studied and hence the term “any fracture” was applied.

In the follow-up study (paper III) fractures of any type (ICD-10 diagnosis codes S12, S22, S32, S42, S52, S62, S72, S82 or S92) and fractures of the hip (ICD-10 codes S720, S721 and S722) were identified (Table 7).

<table>
<thead>
<tr>
<th>ICD-10 code</th>
<th>Location of fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>S12</td>
<td>Cervical vertebra and other parts of neck</td>
</tr>
<tr>
<td>S22</td>
<td>Rib(s), sternum and thoracic spine</td>
</tr>
<tr>
<td>S32</td>
<td>Lumbar spine and pelvis</td>
</tr>
<tr>
<td>S42</td>
<td>Shoulder and upper arm</td>
</tr>
<tr>
<td>S52</td>
<td>Forearm</td>
</tr>
<tr>
<td>S62</td>
<td>Wrist and hand level</td>
</tr>
<tr>
<td>S72</td>
<td>Femur</td>
</tr>
<tr>
<td>S82</td>
<td>Lower leg, including ankle</td>
</tr>
<tr>
<td>S92</td>
<td>Foot and toe, except ankle</td>
</tr>
<tr>
<td>S720</td>
<td>Femur: Collum</td>
</tr>
<tr>
<td>S721</td>
<td>Femur: Pertrochanteric</td>
</tr>
<tr>
<td>S722</td>
<td>Femur: Subtrochanteric</td>
</tr>
</tbody>
</table>

Fractures treated in out-patient care were retrieved from local hospital registers. For identification of fractures in in-patients, the Swedish National Patient Registry (NPR)\textsuperscript{221} was used. Complete fracture identification was achieved by matching the fracture cases in the study cohort with these rec-
ords. Separation of re-admissions of a previous fracture event from incident fracture admissions was done using a previously validated and accurate method\textsuperscript{222}. Retained in the analysis were fractures caused by high-energy trauma, amounting to about 1\% of all fractures. Pathologic fractures caused by malignant disease were not considered as an outcome.

In paper IV, first incident cases of any fracture (ICD-10-codes S02, S12, S22, S32, S42, S52, S62, S72, S82, S92) and first incident cases of hip fracture (ICD-10 codes S720, S721, S722) were ascertained from 1 January 1998 to 31 December 2008 by electronic linkage of the study population to the National Hospital Patient Register and to the Regional Hospital Diagnosis Register.

Genetic analyses (Paper II)

Genotyping of CYP1A2
In the CYP1A2 gene enzyme inducibility is increased by a substitution of C with A at position – 163\textsuperscript{223}. The homozygote carriers of the mutated allele are regarded as "rapid" caffeine metabolisers of caffeine.

Polymorphism of rs762551 in the CYP1A2 gene has been demonstrated to influence the association between coffee intake and myocardial infarction\textsuperscript{71}. However, because this single nucleotide polymorphism (SNP) was later not genotyped in HapMap (http://www.hapmap.org/), another SNP was chosen in HapMap, rs11854147. This SNP is in linkage disequilibrium with rs762551 (\(R^2=0.886\)). The SNP Technology Platform at Uppsala University, Sweden performed the genotyping of the SNP rs11854147 (http://www.genotyping.se).

Low-energy falls (Paper III)

Potential associations between consumption of coffee and fall frequency (at least one fall or at least two falls during the previous year) were examined according to the recordings in the SMCC questionnaire.

Determination of 25-OH vitamin D (Paper III)
The analysis was performed at Vitas, Oslo, Norway (www.vitas.no). One hundred µL of human serum was diluted with 300 µL isopropanol with deuterium labelled 25-OH-vitamin D3 as internal standard. After thorough mixing (15 minutes) and centrifugation (10 minutes, 4000 g at 10°C), an aliquot of 20 µL was injected from the supernatant into the HPLC system.

HPLC was performed with a Agilent 1260/1290 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) interfaced by atmospheric pres-
sure chemical ionisation (APCI) to a Agilent Technologies 6420 Triple Quad LC-MS/MS operated in multiple reaction monitoring mode (MRM).

Vitamin D analogues were separated on an Asentis Express F5 150mm x 4.6 mm column with 2.7 μM particles. CV per cent for 25-OH-Vitamin D (inter-assay variation) was 9.67% (36.94nM) and 10.15% (48.08 nM) using plasma QC samples at two levels analysed in a series with study samples. In the present analysis we used the sum of 25-OH-vitamin D₃ and 25-OH-vitamin D₂ as an estimate of total 25-OH-vitamin D in serum.

Statistical methods and analyses

Cox proportional hazards models (Papers I, III and IV)

To estimate hazard ratios (HRs) with 95% CIs, Cox proportional hazards models were used in papers I, III and IV to measure the association between exposure and the occurrence of osteoporotic fractures. Using Nelson-Aalen plots, the proportionality assumption was verified. For each participant, time at risk was calculated from the date of the baseline questionnaire until the date of fracture, death, emigration or the end of the study period, whichever came first.

In addition, restricted cubic spline Cox regression models were applied in papers I, III and IV to obtain a more limitless view of the shape of the association between the exposure(s) and the risk of osteoporotic fractures.

Statistical analyses in papers I were done by SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). In papers III and IV they were performed by STATA 11 and 12.1, respectively (Stata Corp LP, Collage Station, TX, USA).

Paper I

In paper I, the basic model used to estimate HRs included age at the entry of the study. In addition, the multivariable model included height, weight, intake of vitamin D, vitamin A, calcium, phosphorus, alcohol and energy (all continuous), educational level (low vs. high) and marital status (married or cohabite vs. single or widowed). These variables were included because they could be considered potential covariates. Coffee and tea consumption was assessed in four categories: <1 cup, 1 cup, 2-3 cups and ≥ 4 cups daily. Caffeine intake was assessed in quintiles by the following categories: < 200, 200–209, 210–249, 250–329 and > 329 mg/day.

Fracture risk associated with exposure was separately estimated in a low- and high-calcium group stratified by the mean dietary calcium intake (700
mg/day) of the cohort. This was done because an effect modification by calcium has been indicated in a study of caffeine intake and bone density.

Paper II

In Paper II, analyses of genotyped participants (n = 717) with both dietary assessment and BMD measurement were performed. Adjusted means of BMD at the proximal femur (three sites of measurement) were calculated for each category of coffee consumption. Ordinary models of linear regression were then used to analyse the relation between coffee consumption as a continuous variable and BMD.

Adjustments were made by age (at time of the DXA measurement) or by a multivariable model. The multivariable model included age, height, weight, total caloric intake, intake of vitamin D, vitamin A, calcium, alcohol and tea (all continuous). In the model the categorised variables were smoking (never, current, former) and levels of leisure physical activity (low, medium, high). These variables were included because they appear to be potential confounders. Physical activity was divided into light and hard exercise and classified as number of activities for at least 30 minutes per week. Three physical activity categories were constructed: low, medium and high physical activity.

The statistical analyses in paper II were performed by SAS version 9.1, (SAS Institute Inc., Cary, NC, USA).

Paper III

Because data for several potentially important covariates (smoking status, physical activity, supplementation with calcium and vitamin D, cortisone use and hormone replacement therapy) were missing at baseline in 1987, multiple imputations using the Markov Chain Monte Carlo method were performed in paper III. As in paper I, Cox proportional hazards models were used to estimate crude- and multivariable-adjusted HRs with 95% CIs.

In paper III, the full model included BMI, height, total energy, dietary intake of calcium, vitamin D, retinol, protein, phosphorous and potassium, consumption of alcohol (all continuous), calcium supplementation (yes or no), vitamin D supplementation (yes or no), tea consumption (number of cups per day), educational level (< 9, 9-12, > 12 years and other), physical activity (five categories), smoking status (never, former, current), previous fractures before the study period (yes or no), Charlson’s comorbidity index (continuous, 1-16)²²⁵²²⁶, living condition (living alone or not), cortisone use (yes or no) and hormone replacement therapy (yes or no).

Time-updated variables were used because they take into account changes during follow-up. Consumption of coffee in relation to fracture risk was assessed both as a categorical variable (using the same categories as in paper
I) and as a continuous variable, corresponding to a 200-ml increase (or one cup) of coffee intake. Kaplan-Meier failure curves for the four consumption categories of coffee (< 1, 1, 2-3 and ≥ 4 cups per day) were constructed to display age-adjusted incidences of any fracture and hip fracture in relation to follow-up time.

Logistic regression was performed to analyse the association between coffee consumption and the risk of falls and osteoporosis in the SMCC. Adjusted BMD means for categories of coffee consumption (< 1, 1, 2-3 and ≥ 4 cups per day) were calculated using a general linear model. Using data from the three dietary questionnaires, cumulative averages of coffee consumption and other dietary variables were estimated for the SMCC analyses. Effect modification by calcium on our estimates was investigated by calculating the relative excess risk due to interaction \(^{227}\). Because an intake < 700 mg per day previously had been found to be associated with an increased rate of fractures in our cohort \(^{12}\), this intake was defined as the cut-off for low calcium intake.

**Paper IV**

The same categories of coffee consumption as in Papers I and III (i.e. < 1, 1, 2-3 and ≥ 4 cups daily) were used to analyse the association between coffee consumption and fracture risk in paper IV. In addition, analyses with coffee as a continuous variable (corresponding to a 200-ml increase or 1 cup of coffee) were performed as in paper III.

In a few cases, when data had not been provided by the participants, imputations were performed applying the Markov Chain Monte Carlo multiple method to construct baseline values for several important covariates (e.g., BMI, height, self-reported health, alcohol consumption, education, civil status, physical activity and smoking).

In the analyses in which Cox proportional hazards regression was used, the multivariate model in paper IV included intake of calcium, retinol, vitamin D and alcohol and, tea consumption, BMI, height, physical activity (MET-24h score) (all continuous), intake of any vitamins, cortisone use, educational level (≤ 9, >9 - 12, > 12 years, other), smoking status (never, former, current), previous fractures (yes or no) and Charlson’s comorbidity index (continuous, 1-16) \(^{225,226}\). Several other covariates, initially included in the model, were excluded in the final model because they only marginally affected the associations. As in paper III, Kaplan-Meier failure curves for the four consumption categories of coffee were computed to visualise age-adjusted incidences of any fracture and hip fracture in relation to follow-up.

Additionally, stratified analyses in several categories of age (< 50, 50- < 70 and ≥ 70 years) were performed to assess whether associations were limited to certain age groups.
As in the papers I and III, analyses were performed to determine whether fracture risk might be affected by calcium intake. In this analysis, the cohort was stratified by dietary calcium intake using the median dietary intake (1417 mg/day) in the cohort as the cut-off value between low and high intake of calcium. Furthermore, interactions with calcium were assessed by the inclusion of a product term between coffee consumption and calcium intake in the multivariate model.

Ethics
The regional ethics committee at Uppsala University approved all the studies. In addition, the studies of SMC, SMCC and COSM were approved by Karolinska Institutet, Stockholm.
Results

In this section the main results and conclusions are summarised. A more detailed account is reported in the separate papers (I-IV).

Paper I

In this study, 3,279 cases with first osteoporotic fractures at any fracture site were observed during a total of 321,151 person-years.

The osteoporotic fractures were distributed as follows: 880 fractures of the hip, 524 of the pelvis, 405 vertebral fractures and 1,972 distal forearm fractures and finally 633 fractures of the proximal humerus. In some cases more than one fracture was observed.

Coffee consumption was high in the cohort, with about 59% of the participants reporting a consumption of 2-3 cups of coffee daily and about 19% reporting a daily intake of ≥ 4 cups (600 ml) of coffee.

With increasing coffee drinking, a statistically significant linear trend for increased risk of all fractures was found (p for trend = 0.002). Participants consuming ≥ 4 cups of coffee per day compared with those consuming < 1 cup/day in the low calcium intake sub-cohort had a HR of 1.33 (95% CI 1.07 - 1.65). For women with higher propensity of fractures, i.e. those who had suffered two or more fractures (n= 736), the corresponding HR was further strengthened in the low calcium group: (HR = 1.88; 95% CI 1.17 - 3.00) but not in the high calcium intake group: (HR = 0.89; 95% CI 0.53 - 1.45).

No statistically significant associations between consumption of tea and incidence of osteoporotic fractures were observed.

A statistically significant increased risk of fracture (HR = 1.20; 95% CI 1.07 - 1.35) was found in women in the highest quintile of caffeine intake (median intake 350 mg/day) compared with women in the lowest quintile of intake (median intake 100 mg/day). However, the increased risk for any osteoporotic fracture was statistically significant only when calcium intake was low (< 700 mg/day). The multivariate-adjusted HR of the highest compared with the lowest quintile of caffeine intake was 1.28 (95% CI 1.09 - 1.50) at low calcium intake. The overall trend was statistically significant (p = 0.003).

When the analyses were restricted to women with two fracture types or more, those with a low calcium intake within the quintile with highest caf-
feine intake had a higher HR (HR = 1.49; 95% CI 1.13 - 1.96) compared with those with a low calcium intake in the lowest quintile of caffeine intake.

As indicated in Figure 12 (restricted cubic spline Cox regression model), the risk of osteoporotic fractures is significantly increased by an intake between 300 and 350 mg caffeine daily, corresponding to the highest quintile of caffeine intake in the cohort or ≥ 4 cups (600 ml) of coffee.

In conclusion, an increased risk of osteoporotic fractures in individuals consuming ≥ 4 cups of coffee in comparison with those consuming < 1 cup coffee per day was observed in a study of 31,257 Swedish middle-aged and elderly women when calcium intake was low (< 700 mg/day). In addition, an increased risk of fracture was found in women in the highest quintile of caffeine intake (median intake 350 mg/day) compared with women in the lowest quintile of intake (median intake 100 mg/day). However, this increased risk for osteoporotic fracture was statistically significant only when calcium intake was low. Tea consumption at any level did not affect the risk of osteoporotic fractures. Data on important confounders (such as smoking, physical activity and use of oestrogen) were lacking in this study. The consequences of nutritional habits and high risk of fractures of many elderly women may, according to the results of this study, be important for public health.
The reported consumption of coffee was 3 or 4 cups per day in half of the participants of the PIVUS cohort and > 4 cups of coffee daily in one fourth of the cohort.

There was a trend of decreased BMD at the total proximal femur (p for trend = 0.04) with increasing amounts of coffee consumed after multivariable adjustment. In comparison with men drinking 0 - 2 cups of coffee/day, men drinking ≥ 4 cups of coffee/day had a 4% lower BMD at the total proximal femur (p = 0.04). This difference was not observed in the female participants. Lower BMD values (approximately 2- 4%) were found in rapid metabolisers (both sexes) of caffeine (Figure 13). These differences attained statistical significance at the femoral neck (p = 0.01) and trochanter region (p = 0.03), but not at the total proximal femur (p = 0.10). Adjusted average BMD was not higher in high consumers of coffee (≥ 4 cups/day) with a high calcium intake (> 1200 mg/day) when compared with those with a lower calcium intake (<600 mg/day). There was no association between tea consumption and multivariable-adjusted BMD.

In summary, a 4 % reduction in BMD of the proximal femur was found in male participants of the PIVUS cohort with high consumption of coffee (≥ 4 cups of coffee/day vs. 0 - 2 cups/day). In addition, in participants with high

![Figure 13. Mean-adjusted BMD of the total proximal femur in men and women with a high consumption of coffee (≥ 4 cups per day) by CYP1A2 polymorphism. The error bars indicate 95% confidence intervals (CIs) and the p-values refer to comparisons between slow and rapid metabolisers at each site. (Adapted from Figure 1, Paper II).](image-url)
coffee intake, those who were rapid metabolisers of caffeine had a 2 - 4% lower BMD than slow metabolisers. It is possible that these individuals may constitute a potential risk group for developing osteoporosis. More studies are needed to confirm these results.

Paper III

A daily consumption of 2 - 3 cups of coffee was reported by approximately 60% of the participants of the SMC and 18% consumed ≥ 4 cups of coffee. During a median follow-up of 19.4 years, 24% of the cohort (i.e. 14,738 women) experienced a first fracture at any site. Of these first fractures, 3,871 were hip fractures.

There was no evidence of a higher rate of any fracture (HR = 0.99; 95% CI 0.98 - 1.00 per 200 ml coffee) or hip fracture (HR = 0.97; 95% CI 0.95 - 1.00 per 200 ml coffee) with increasing coffee consumption. In comparison with women with a low intake (< 1 cup), women consuming ≥ 4 cups of coffee daily had a HR of 0.96 (95% CI 0.90 - 1.02) for any type of fracture and a HR of 0.88 (95% CI 0.78 - 1.00) for hip fracture.

Not even at a very high coffee consumption (≥ 8 cups per day) was there an increased risk of any fracture (HR = 0.95; 95% CI 0.80 - 1.14) or of hip fracture (HR = 1.20; 95% CI 0.82 - 1.75). Calcium intake did not affect these results, i.e. no effect modification was observed.

High (≥ 4 cups) vs a low (< 1 cup) coffee consumption, however, was associated with a 2 - 4% lower BMD, depending on site (p < 0.001) (Figure 14), but there was no increase in the risk of osteoporosis (OR = 1.28; 95% CI 0.88 - 1.87). The rate of low-energy falls in the sub-cohort (SMCC) was not associated with coffee consumption.

To sum up, in study III high coffee consumption (≥ 4 cups vs. < 1 cup) was associated with a modest reduction in BMD. However, this decrease in BMD did not translate into an increased risk of osteoporosis or fractures.
Figure 14. BMD at the spine, proximal femur and in the total body in relation to coffee consumption.

Paper IV

During a median of 11.3 years of follow-up (totally 483,508 person-years), a first fracture at any site was reported by 5,066 participants in COSM, corresponding to about 12% of the cohort. In the total number 1,186 were hip fractures (approximately 3% of the cohort).

Consumption of coffee was high in this cohort: about 40% of the cohort consumed 2 - 3 cups daily and another 40% reported a consumption of ≥ 4 cups per day. Tea consumption was low. Coffee and tea consumption were inversely related. In accordance with results from previous studies, smoking was associated with a high consumption of coffee.

In Figure 15 age-adjusted incidences for any fracture and hip fracture by category of coffee consumption are displayed. No statistically significant associations between consumption of coffee (≥ 4 cups vs < 1 cup per day) and risk of any fracture (HR = 0.91; 95% CI 0.80 - 1.02) or hip fracture (HR = 0.89; 95% CI 0.70 - 1.14) were observed after multivariate adjustment. Further, no threshold could be identified, i.e. there were no associations even
at the extremely high consumption of ≥ 8 cups in comparison with < 1 cup per day (data not shown). This finding held when coffee consumption was assessed as a continuous variable. HR for any fracture was 1.00 (95% CI 0.99 - 1.02) and for hip fracture 1.02 (95% CI 0.99 - 1.06) per 200 ml of coffee after multivariate adjustment. However, among men aged ≥ 70 years (constituting 22% of the cohort), a tendency for increased rate of hip fractures with very high intakes of coffee consumption could be observed. Finally, in this study no effect modification by calcium intake could be demonstrated.

No non-linear associations between coffee consumption and incidences of any fracture or hip fracture could be detected in the restricted cubic-spline Cox regression analyses.

In conclusion, no association between increasing coffee consumption and fracture risk was detected in COSM during the follow-up.
Figure 15. Age-adjusted incidences for any fracture (A) and hip fracture (B) by category of coffee consumption in the Cohort of Swedish Men (COSM)
Discussion

The overall aim of this thesis was to investigate whether long-term consumption of coffee, tea or intake of caffeine is associated with adverse effects on bone health in men and women. The ultimate goal is to improve the scientific basis for dietary advice regarding consumption of coffee and tea to individuals at risk for osteoporosis and fractures. Since a large proportion of the population is likely to be affected by these outcomes, the present results are important for general public health.

Coffee, tea, caffeine and fractures (Papers I, III, IV)

Previous studies
Studies investigating an association between consumption of coffee/intake of caffeine and risk of osteoporotic fractures in women have produced conflicting results. In most of these studies no association between this exposure and osteoporotic fractures could be found, whereas an association was observed in some of the studies. For men, most studies have not demonstrated increased risk for osteoporotic fractures with increasing consumption of coffee or intake of caffeine.

However, many of the studies with female participants were relatively small with short follow-ups and few fracture cases. In addition, control of lifestyle habits was often inadequate, consumption of coffee was relatively low and the exposure range was narrow. Moreover, consumption of tea and coffee was not considered separately in some studies. Essentially the same limitations previously described in the studies of women were often reported in the studies with male participants.

Paper I
In Paper I, it was found that participants who were a part of the SMC (n=31,527) and whose caffeine intake was equivalent to approximately ≥ 4 cups (600 ml) of coffee per day had an increased risk of osteoporotic fractures when their calcium intake was low (< 700 mg/day). Tea consumption at any level did not influence the risk of osteoporotic fractures in this study.
Some human metabolism studies have suggested that caffeine increases urinary calcium excretion and decreases intestinal calcium absorption efficiency. These mechanisms might result in promotion of a negative calcium balance, which could be important for bone loss, especially when intake of caffeine is high and ingestion and absorption of calcium is low. A modifying effect of calcium intake on the risk of fracture associated with a high consumption of caffeine has also been indicated in studies with BMD as outcome. The results of paper I suggest that the possible modification on fracture risk by calcium intake should be considered in studies of this kind.

The strengths of this study include the prospective longitudinal design, the large number of participants, the long follow-up (10.3 years) allowing detection of an adequate number of osteoporotic fractures, the good case ascertainment by use of X-ray diagnosis, the wide range of consumption of coffee consumed in this cohort and that focus was not only on caffeine intake but also on the exposure of coffee and tea separately.

During the investigation, we had the possibility to include only one of the counties of the SMC, because one county (Västmanland) did not have a complete out-patient diagnosis code reporting system. Complete coverage of out-patient treated fractures from 1987 was later possible by the combination of x-ray reports, scrutinising hospital records of cohort members and use of local computerised diagnosis systems.

A limitation of the study is that there may be some error in the exposure measurement because it is based on a single FFQ. Another limitation is that analytical data are lacking on caffeine concentrations in the coffee and tea. This limitation results in difficulties in estimating the participants’ actual intake of caffeine. On the other hand, it is well-known that coffee and tea are beverages containing several other bioactive compounds that may be important for bone health. Thus, focusing on caffeine only may not be altogether relevant. Information about supplements that can affect fracture risk was missing.

The most important limitation in paper I is that data on smoking and physical activity, which are important lifestyle characteristics in this context, were lacking. Still, no substantial effects on the fracture risk estimates were observed in some cohort studies having quantified and adjusted for smoking in their coffee and caffeine analyses. In addition, an adjustment was performed for total energy intake that can be regarded as a proxy variable for physical activity, since it is known that high-energy intake has been associated with higher physical activity.

Paper III

In paper III, the entire SMC (including 61,433 women) was studied for consumption of coffee and incidence of osteoporotic fractures. In addition, cof-
Caffeine consumption in relation to BMD was investigated in the SMCC. (see Coffee and BMD below).

In study III it was found that a high daily long-term coffee consumption (equivalent to ≥ 4 cups) was associated with only a slightly lower BMD. The small difference in BMD was not translated into a higher rate of fractures or risk of osteoporosis and no significant association between coffee consumption and low-energy falls was observed. Furthermore, the relation between coffee consumption and fracture incidence was not influenced by calcium intake. Thus, the previously found effect modification by calcium in paper I was not confirmed in paper III, where time-dependent exposure and covariate information were considered.

The major strength of study III is that we were able to collect a large number of fractures (n = 14,738) with complete coverage of the cases and that a study of BMD as a secondary outcome could be performed in a large sub-cohort. Another important strength in study III is that, whereas results were based on the first FFQ only in paper I, repeated FFQs were used in paper III that allowed the use of time-dependent exposure. This is likely to have contributed to a reduction of measurement error in the exposure information. Moreover, data on smoking, use of HRT and physical activity, that may have influenced the obtained estimates, were included in the adjustments in the multivariate analyses of study III. Finally, a high and wide range of coffee consumption constitutes an additional strength of study III.

Some limitations in paper III should be mentioned (see also: General methodological considerations below). Although there are validation data suggesting that the coffee consumption reported in the questionnaires is a reasonable estimate of the exposure, it is inevitable that using self-administered FFQs results in measurement errors. It could be questioned whether fractures that were due to high trauma should be included in the analyses of osteoporotic fractures, but because a comparable increased risk of both low- and high-trauma fracture with decreasing bone density in the elderly have been indicated 229, they were retained in the analyses. Finally, we were not able to take genetically derived differences in the metabolism of caffeine into account in this study, although we cannot exclude the possibility that they might be of relevance in this context 230.

Paper IV
As in the SMC, we did not find any significant associations between consumption of coffee and incidence of fractures in the COSM. An exception might be a slight increase in the risk of hip fracture among elderly men (≥ 70 years) who consumed large amounts of coffee. However, the importance of this finding is uncertain because of the low precision in these estimates. Furthermore, the results reported in paper IV were not affected by calcium in-
take. The overall result of this study is in accordance with the results obtained in previous epidemiological research in men.

The major strengths of this investigation are the large population-based cohort of middle-aged and elderly participants, the 11.3-year follow-up, the generation of a large number of fractures (n= 5,066) identified by registers and the high and varied consumption of coffee in the cohort.

There are potential limitations of this study. First, exposure data are derived from a single FFQ and therefore error in the exposure measurement cannot be ruled out. This could have resulted in an attenuation of true associations because the potentially resulting misclassification probably would be non-differential. Like in the SMC, fractures associated with high trauma were not excluded in the analyses. Such an analysis might result in a lower risk estimate for all analysed fractures than for low-trauma fractures only\textsuperscript{231}.

**Meta-analysis**

In this context, a recent meta-analysis on coffee consumption and risk of fractures may be relevant\textsuperscript{232}. In this meta-analysis 10 prospective cohort studies, including the study reported in paper I were included. The studies covered 214,059 participants and 9,597 fracture cases. For each increase in the number of cups of coffee consumed daily, the overall fracture risk was 3.5% (RR = 1.035; 95% CI 1.019 - 1.052) according to the results from the cumulative meta-analysis. Taking sex into account, the RR for women was 1.049 (95% CI 1.022 - 1.077) and 0.910 for men (95% CI 0.873 - 0.949) for each increase in the number of cups of coffee consumed daily.

Thus, this meta-analysis indicates an association between increasing coffee intake with a concomitant increased risk of fractures, though separate analyses of men and women, suggest that it was confined to women. However, it is likely that publication bias that results in the more frequent publication of studies producing significant results may have affected the results of this meta-analysis. Another problem may be the occurrence of residual confounding. The authors concluded that current data are insufficient to draw any firm conclusions and that further research is needed.

**Coffee and bone mineral density (Papers II and III)**

**Previous studies**

In numerous studies in men no statistically significant associations have been observed between consumption of coffee and BMD/BMC or between caffeine intake and BMD. The results of studies investigating these associations in women are not consistent. In a few studies associations between
consumption of coffee and BMD/BMC or between caffeine intake and BMD have been observed, but in general, the studies in women have only provided limited evidence for the existence of such associations. The lack of association in the studies might be explained by small sample size, low intake of coffee/caffeine and that no separate analyses of coffee and tea were performed.

Paper II

In paper II, participants in the cohort of PIVUS were investigated. In this study a decrease of 4% in BMD of the proximal femur was observed in men consuming ≥ 4 cups of coffee compared with low or non-consumers. No difference in BMD between high and low consumers of coffee was noted in women. Moreover, in high coffee consumers rapid metabolisers of caffeine had lower BMD values at the femoral neck and at the trochanter than slow metabolisers of caffeine. It is thus possible that a potential risk group for developing osteoporosis has been identified. The relation between coffee and BMD was not affected by calcium intake.

It seems that caffeine will be more rapidly metabolised by men, probably because the activity of CYP1A2 is higher in men. This will result in higher concentrations of metabolites, like paraxanthine, relative to the concentration of caffeine. It may be hypothesised that caffeine metabolites are responsible for the deleterious effect of coffee consumption on bone. Consistent with this theory is our observation of a lower BMD among rapid metabolisers in comparison with slow metabolisers of caffeine with high coffee consumption.

Caffeine and paraxanthine are teratogenic after administration of very high doses in mice. Typically, skeletal malformations develop. Paraxanthine in vitro has been reported to be a potent suppressor of transforming growth factor beta (TGF-β). TGF-β is known to stimulate bone formation and deficiency in TGF-β may result in osteoporosis.

Paraxanthine, as well as other major caffeine derivatives, have common mechanisms of action, i.e. competitive antagonism of the adenosine interaction with A1 and A2 receptors. In bone cells the deactivation of the adenosine receptors can result in reduced bone formation.

The strengths of paper II include a prospective design, a wide range of coffee consumption, a sufficient number of participants with a high consumption, a sufficient number of participants to detect even a modest association and separate analyses of the exposure of coffee and tea. An additional strength is the investigation of the possible modification of BMD by genotype for CYP1A2 inducibility, which has not been investigated previously.

However, potential limitations should also be addressed. It might be questioned that the measurement of BMD was performed in the proximal femur only. The reason for not including BMD measurements of the spine is that
spondylosis is common in elderly individuals. This condition can confound the relative weak association between BMD and coffee as well as the comparison between the sexes. Another potential drawback is that follow-up was limited to 2 years. However, the optimal time between measurements of coffee consumption and BMD is not known at present.

Regarding the genotype for CYP1A2 inducibility, it has also been questioned whether the C allele at position -163 in the CYP1A2 gene, thought to confer decreased inducibility to the CYP1A2, is associated with activity of the enzyme. (Comment in Nutrition and Metabolism 2011-06-21 by Perera V. at the University of Sidney, Australia 237.) According to this comment, CYP1A2 polymorphism (-163C>A) is only associated with higher inducibility in smoking individuals. This is, however, a matter of debate since it is known that, in addition to smoking, certain drugs and a number of dietary factors such as coffee 223, cruciferous vegetables 238 and PAH 239 may have an inducible effect upon the activity of CYP1A2.

In the investigation of inducibility of CYP1A2 and BMD, statistical significant differences in BMD between high consumers who were rapid metabolisers and those who were slow metabolisers of caffeine were generally restricted to the whole study group of both men and women, presumably as statistical power was too low to attain statistical significance in the groups of each sex.

Another limitation of the study is that some degree of error in the exposure assessment is likely in the sense that the exposure measurement was based on a single dietary measurement. Because caffeine content in the consumed coffee and tea was not analysed, data on the actual intake of caffeine are lacking. It is, however, known that recall errors often result in estimates that are conservatively biased.

Finally, it is possible that other mechanisms than reduced renal calcium conservation may explain some of the effects of caffeine or its metabolites on bone. To clarify possible mechanisms of interactions in caffeine intake and CYP1A2 genotype in relation to BMD, more studies are recommended.

Paper III
In the sub-cohort SMCC a high daily long-term coffee consumption, equivalent to ≥ 4 cups of coffee compared with < 1 cup daily, was associated with a slightly lower BMD. In study III, however, the small differences in BMD associated with coffee consumption did not affect the risk of osteoporosis, although a weak tendency (but not statistically significant) toward an increased risk of osteoporosis with higher coffee intake was noted.

The decrease in BMD was, on average, 4% in the lumbar spine and 2% in both the proximal femur and in the total body. The results of this study thus contradict the results for women in paper II. Nevertheless, the power of study II, which included about 300 women, was considerably lower than that
of study III, which comprised over 5,000 women. Furthermore, BMD data are not readily comparable in that only BMD of the proximal femur was investigated in study II.

As with the outcome osteoporosis, the modest reductions in BMD with increasing coffee consumption observed in this study did not have an impact on the risk of incident fractures.

Possible explanations for this result may be that the small decrease in BMD observed with high coffee consumption could be counteracted by reduced probability of hypotension and comorbidities, resulting in a reduced predisposition of injurious falls. Thus, there would be no excess risk of fractures.

Coffee and risk of falling

Compared with factors related to accidental falls, the impact of a decrease in BMD on hip fracture rate has been found to be more modest in the oldest-old (often defined as older than 85 years) compared with the young-old (often defined as 65 – 75 years old).

However, in this study no association between coffee consumption and risk of accidental falls was demonstrated. An explanation might be that self-reported falls were not accurately reflected by actual injurious falls.

Influence by vitamin D status on the relation between coffee consumption and BMD

Contrary to prediction, a somewhat stronger association between coffee intake and a decrease in BMD was detected among women with 25-OH-D values higher than 50 nmmol/L, which was the cut-off between high and low vitamin D status.

A reason for this result may be that low vitamin D status is likely to be associated with frailty. Among the participants with low 25-OH-D values, the association between coffee consumption and bone density might not be easily detected when other factors related to frailty that more strongly influence BMD are present.

General methodological considerations

Unlike the situation in randomised clinical studies, it is not possible to conclude whether associations in observational studies are causal, because the participants in observational studies are not randomised to the exposure variable.

In epidemiological research two types of error can occur, namely random and systematic. Random errors arise due to sampling variability or lack of measurement precision. Such errors can be minimised, but cannot be completely eliminated. Sample size and the quality of the data determine the
precision of a study. If random errors are absent, precision is high. Statistical analysis can reveal and address random errors, whereas most systematic errors cannot be identified and corrected in this way.

Systematic errors or biases, on the other hand, occur when a false pattern of differences between observed and true values arise that are not due to variability in sampling. Systematic errors may originate from methodological errors, including complex human factors. Avoiding systematic errors or biases may be facilitated by a suitable research design. Minimising systematic errors is important to improve the internal validity of a study.

Study design
The studies presented in this thesis are all population-based prospective cohort studies and should as such constitute representative samples of the general population in terms of age, sex, nutritional factors, lifestyle, socio-economic factors and incidences of diseases. The investigation of the effects of coffee consumption and risk of fracture should ideally be evaluated in a controlled randomised study. However, the feasibility to conduct such studies is limited because of methodological challenges (e.g., study size, compliance, drop-outs, blinding and long-term follow-up). Therefore, it is not surprising that there are no randomised trials in this area. The drawbacks of the available study design have been addressed in the statistical analyses by adjustment of confounding factors as far as possible. Moreover, the impact of the lack of a control group is reduced by the wide range of exposure i.e. the intake of coffee ranges from low (approaching placebo) to high consumption.

In a prospective cohort study a large number of participants may be followed during a long period, which will be an advantage when studying common diseases with long latency. Furthermore, a prospective design ensures that the temporal sequence between exposure and outcome is appropriate in that exposure is determined before the outcome is observed.

Selection bias
Selection bias is a type of systematic error that may be introduced when there is a difference between individuals selected for participation in a study and those individuals not selected, or when other factors exist that will affect participation or follow-up.

In prospective studies, differential loss to follow-up can introduce selection bias, i.e. bias may arise if an incomplete follow-up is different in different exposure categories. However, because we could use computerised linked data from the National Patient Registry and regional fracture registries, the follow-up of fractures should be almost complete, which markedly reduces the possibility that results were biased by differential follow-up.
In the SMC the age range was rather wide (at start in 1987: 39 - 73 years). Such a range might have resulted in selection bias in that older individuals might have been less healthy than younger ones and thus less prone to participate. On the other hand, younger individuals might not have the time needed for participating in investigations of this kind, which is likely to have reduced their chances to participate.

Moreover, because the BMD measurements were included in the investigations of the SMCC, it cannot be excluded that individuals participating were healthier than those who did not participate because these latter individuals might have been hampered by limitations in their mobility due to osteoporosis. This hypothesis, however, seems less probable because a similar prevalence of osteoporosis in Swedish women has been reported. Moreover, in terms of covariate exposure, a homogenous cohort with a sufficient exposure range can be regarded as an asset of the study.

Information bias
Information bias may result from incorrect determination of exposure or disease outcome, or both. In a cohort study information about outcomes should be obtained in the same way for the exposed as for the unexposed participants. This kind of bias occurs when measurement of information on exposure or disease differs between the study groups or is inaccurate independent of exposure categories leading to attenuated associations. A major strength of our studies on fracture as outcome is that we focused on time to first incident fracture. Changes in lifestyle due to the fracture event were therefore minimised.

Misclassification regarding exposure and outcome may be differential or non-differential. In the former the measurement errors are related to the disease or exposure, whereas in the latter there is no relation between measurement errors and the disease or exposure. If there were no difference in misclassification between the exposed or unexposed or with or without the outcome, the result would be an underestimation of the strength of the association. In other words, a non-differential misclassification would tend to mask real differences. In this context the older participants in the investigated cohorts might have changed their habits of coffee consumption as a consequence of prevalent illnesses. Therefore, in addition to age, we considered a comorbidity index in some, but not in all, of our analyses.

Misclassification of exposure
Using FFQs is often associated with measurement error because the nutritional data obtained from FFQs are derived from self-reported intake of certain foods. When using this method for exposure measurements, participants tend to over- or underestimate their food consumption. To detect such problems validation of the FFQ is essential.
In the SMC a validation of the FFQ used at baseline was performed. The validity of the FFQ in comparison with dietary records was found to be high. In COSM, validation of the FFQ (the same FFQ used in SMC, 1997) was performed. The FFQ was validated for macro- and micronutrients, but not for food items in COSM. A high validity in comparison with the reference method was demonstrated for macro- and micronutrients.

**Special consideration regarding estimation of coffee and tea intake in the investigated cohorts**

Because estimation of coffee and tea intake relies on the type of FFQ used, discussing potential problems with classification of these exposures is essential. Not all FFQs used in the studies provided definitions of cup size. But even if the cup size would have been defined, data on how participants selected (type and brand), prepared (in case of coffee, brewing, boiling or percolating) and drank their coffee/tea (with and without milk) are not available from the studies. These circumstances are also likely to have affected the exposure to the bioactive compounds in these beverages.

Such shortcomings are, however, inevitable in most epidemiological studies involving coffee and tea as exposure variables. Most important is that the study of dose-response relationships for coffee/tea exposure, with reference to bone health parameters, can be performed in the investigated cohorts.

**Misclassification of outcome**

Reassessments of BMD, both by triple measurements in 15 individuals and by daily repeated spine phantom measurements, indicated high precision. In addition, misclassification in BMD is less likely to have occurred because of the consistency in the results between the different skeletal sites being measured. However, importantly, the individual BMD measurement by DXA is suggested to be subject to sizable inaccuracies, the magnitude of which can, at its worst, be several T-score units at the lumbar spine and cannot be reliably remedied by any means. The clinical significance of these inaccuracies for the individual patient can be substantial, but in a cohort analysis when analysing an exposure variable in relation to BMD, the impaired validity is of less importance. In papers I, III and IV, misclassification of data for the disease outcome (i.e. fractures) is likely to be minimal because this information was obtained from registries with almost 100% case ascertainment.

**Confounding**

Confounding means that an association between exposure and outcome in a study is mixed or blurred by a third factor. In the multivariable analyses performed in the studies, potential confounders considered were associated with the exposure and the outcome, as well as accepted risk or protective factors.
for the outcome. Important confounders in this thesis include age, smoking, intake of vitamins A and D and calcium, morbidity, physical activity, use of hormone replacement therapy and socio-economic factors.

In addition, suffering a previous fracture is a strong risk factor for subsequent fracture. On the other hand, a fracture could affect the probability of being more inclined to change one’s lifestyle.

Finally, it must be emphasised that residual confounding cannot be excluded in the studies reported in this thesis, even though the statistical models used adjusted for a variety of potential confounders.

**Generalizability (external validity)**

The study bases of the large cohorts SMC and COSM are found in the general population of Central Sweden. The participation rate was high (74%) in the SMC, indicating that it should be possible to generalise the results to the middle-aged and elderly female Swedish population. The participation rate in the COSM was considerably lower (49%). Yet, according to a validation study, the participants are representative of Swedish men for age range, distribution of age, educational level and prevalence of overweight. Finally, generalizing the results for the observed associations between CYP1A genotype and BMD from the PIVUS cohort, despite low number of participants and low participation rate (49%), is not likely to be a serious problem because the frequency of participation probably does not affect the distribution of the genotypes.
Conclusions

High coffee consumption and high intake of caffeine were in our first study associated with increased risk of fractures in middle-aged and elderly women with low intake of calcium (Paper I).

In subsequent studies, however, there was no evidence of higher rates of fractures with increasing consumption of coffee in middle-aged and elderly women (Paper III) or in men of the same ages (Paper IV). These findings held regardless of low calcium intake.

No relation between consumption of tea and incidence of fractures was observed in women, but the setting was not optimal for the investigation of these consumers (Paper I).

A high intake of coffee was associated with a modest decrease in BMD in men (Paper II) and women (Paper III), but no increased incidence of osteoporosis or rate of low-energy fall could be demonstrated in the women (Paper III).

In high consumers of coffee, those with rapid metabolism of caffeine had lower BMD at the femoral neck (Paper II). These individuals may constitute a potential risk group for developing osteoporosis from high coffee intake, but confirmation of the finding is necessary.

In the majority of the studies calcium intake did not modify the relation between coffee intake and BMD, risk of osteoporosis or fractures (Papers II, III, IV).
Future research

The research in this thesis has increased our knowledge about the association between coffee/tea consumption and potential effects on bone health in the Swedish population.

Future research should concentrate on the following:

Clarify possible mechanisms of coffee and tea on bone in in vivo studies.

Elucidate the importance of genetic polymorphism in the caffeine metabolism on bone health. Future research needs to clarify whether CYP1A2 polymorphism modulates the association between coffee intake and fracture risk.

Explore the association between tea consumption and fractures and BMD in populations with high consumption of tea.

Investigate potential differences between black and green tea with respect to parameters related to bone health.

Perform an updated meta-analysis in order to summarize the evidence from all currently available studies and to evaluate potential dose-response relations between consumption of coffee/tea and bone health.
Osteoporos (benskörhet) är en systemisk skelettsjukdom som leder till skörrare skelett och ökad risk för frakturer. Det beror på att benvävnaden har reducerats och fått förändrad mikrostruktur. Tidigare har osteoporos enbart betraktats som ett tillstånd som kliniskt kunde manifesteras i form av frakturer och inte som en sjukdom i sig.


Personer med osteoporos drabbas ofta av så kallade lågenergifrakturer dvs. benbrott efter påfrestningar som ett normalt skelett klarar av. Som osteoporosrelaterade frakturer räknas vanligen frakturer i höft, handleder, underarmar och ryggkotor. I detta sammanhang är det dock viktigt att påpeka att många frakturer som inträffar vid dessa lokalisationer i skelettet ofta förorsakas av fall och inte enbart av benskörhet.

För att diagnostisera osteoporos mäter man bentätheten, oftast med hjälp av en speciell röntgenteknik, som benämns DXA. Man väger också in kliniska parametrar, som genomgångna frakturer och andra relevanta riskfaktorer för fraktur samt gör en bedömning av rörelseorganens funktion, inklusive rygg och leder.

Exempel på starka riskfaktorer för osteoporos/osteoporosrelaterade frakturer är hög ålder, ärftlighet för osteoporos, tidigare frakturer och låg bentäthet. Benägenhet för att falla är också en mycket väsentlig riskfaktor. Att träna upp muskelstyrkan och balansen och att minska medicineringen med sedativa har visat sig vara väl fungerade preventiva åtgärder mot fall. Att dagligen motionera och vid hög ålder använda speciella höftskydd för att förhindra höftfrakturer kan också reducera risken för att drabbas. Det är
också känt att ett flertal livsstilsfaktorer som t.ex. matvanor, intag av alkohol, fysisk aktivitet, rökning och exponering för solljus har betydelse för risken att drabbas av osteoporos, vilket innebär att det också finns möjlighet att i viss utsträckning påverka sjukdomens uppkomst.

Det är inte enbart bentätheten som avgör om en person med osteoporos bör behandlas med läkemedel. Snarare är det personens totala risk för att drabbas av frakturer som avgör behovet. För personer med osteoporos är det därför av stor vikt att försöka minima mina om antalet riskfaktorer.

I likhet med andra livsstilsfaktorer - exempelvis fysisk aktivitet, användning av tobak respektive alkohol - kan också matvanor ha samband med bentäthet och risk för fraktur till följd av osteoporos. Kosten har betydelse, eftersom felaktiga matvanor kan leda till otillräcklig tillförsel av energi och näringsämnen och även orsaka brist på vitaminer och mineralforsörjningen. Speciellt i fokus när det gäller kostfaktorer har intag av vitamin A, D, kalcium och protein varit.


Dessutom har det visat sig i studier på friska, frivilliga personer och även i försök på djur att intag av koffein kan leda till något försämrad kalciumbalans, vilket beror på försvårat upptag av kalcium i tarmen och ökad utsändning av kalcium via njurarna. Hur stor denna effekt egentligen kan bli i praktiken har dock diskuteras, men det är möjligt att den kan ha viss betydelse om man har ett mycket högt koffeintag och samtidigt lågt kalciumintag. Resultat från vissa epidemiologiska undersökningar tyder också på att denna mekanism kan vara intressant. Genetiska skillnader kan troligen också spela roll. Individer med en viss genetisk variant av vitamin D-receptorn förefaller enligt en studie vara mer känsliga för koffeins effekter på benvävnaden. Som framgår nedan, kan även genetiska skillnader i metabolismen av koffein tänkas spela roll i detta sammanhang. Det är dock fortfarande inte klarlagt vilken eller vilka av dessa mekanismer som har störst betydelse eller om det finns ytterligare mekanismer av intresse.

Eftersom vi i Sverige, i internationell jämförelse, har mycket hög konsumtion av kaffe och samtidigt mycket hög incidens av osteoporos är det viktigt från ett folkhälloperspektiv att klarlägga om det kan finnas några samband.

Målet för denna avhandling har därför framför allt varit att undersöka om det finns några samband mellan hög konsumtion av kaffe och bentäthet re-
spektive osteoporos och osteoporosrelaterade frakturer i några av de stora svenska kohorter (undersökningssgrupper) som följts under en längre tid.

De kohorter som här varit aktuella är den svenska mammografikohorten – Swedish Mammography Cohort (SMC), Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) samt Cohort of Swedish Men (COSM). SMC omfattade, då den startades, 61 433 kvinnor som hade rekryterats i samband med att de deltog i mammografiundersökningar. Under den 19 år långa uppföljningstiden av SMC uppstod mer än 14 000 frakturer. I vår studie av COSM ingick knappt 43 000 män med en uppföljningstid på 11 år och under denna tid rapporterades ca 5 000 frakturer i kohorten. I PIVUS undersöktes drygt 700 personer (hälften män och hälften kvinnor) under ca två år.

I uppföljningarna ingick bland annat enkäter med frågor om kost (inklusive intag av kaffe och te) och andra livsstilsrelaterade faktorer. Uppkomna frakturer hos deltagarna klassificerades av ortopedisk expertis och kontrollerades med hjälp av sjukvårdens register. Dessutom gjordes bentäthetsmätningar på en del av mammografikohorten i en s.k. subkohort (Swedish Mammography Cohort Clinical – SMCC) som omfattar 5 022 kvinnor.

Eventuella samband mellan intaget av te och osteoporosrelaterade frakturer har också undersömts. Dessutom har vi kunnat studera om intaget av kalcium och vitamin D från kosten kan påverka eventuella funna samband. Om genetiska skillnader i metabolismen av koffein kan påverka eventuella samband mellan intag av kaffe och bentäthet har också studerats.

De studier som ligger till grund för denna avhandling har visat att det både hos män och kvinnor finns ett samband mellan högt intag av kaffe och något lägre bentäthet vilket, som framgått ovan, kan vara biologiskt rimligt och som stöds av vissa tidigare studier. Men skillnaden i bentäthet var marginal och trots att detta rör sig om mycket stora studier med ett stort antal frakturfall, kunde ingen statistiskt belagd ökad risk för fraktur eller osteoporos upptäckas. Något samband mellan konsumtionen av te och frakturrisken kunde inte observeras, men det ska tilläggas att den svenska tekonsumtionen internationellt sett är mycket låg.


Vi har också visat att högt kaffeintag hos individer med snabb metabolism (omvandling) av koffein kan påverka bentätheten negativt. Det är möjligt att
sådana individer kan utgöra en speciell riskgrupp för negativ påverkan på benvävnaden, men då detta är ett helt nytt fynd krävs uppföljande studier för att bekräfta detta samband. Däremot förefaller inte nivån på intaget av kalcium ha någon betydelse för de funna sambanden. Inte heller påverkas risken av fall, som är en mycket viktig riskfaktor för frakturer hos äldre, av hög kaffekonsumtion.

Sammanfattningsvis har de studier som ligger till grund för denna avhandling visat att hög kaffekonsumtion kan ha samband med en viss sänkning av bentätheten, men inte med förhöjd risk för osteoporos eller osteoporosrelaterade frakturer.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.