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# Fractures in postmenopausal women

Background factors and fracture risk assessment

Louise Moberg



**LUND**  
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DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.  
To be defended at the Department of Obstetrics and Gynaecology.

Friday October 14, 2016 at 13:00.

*Faculty opponent*

Professor Karl Michaëlsson

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Title and subtitle: Fractures in postmenopausal women - Background factors and fracture risk assessment		
<p>The aim of the included studies is to investigate risk factors for fractures in postmenopausal women and explore whether a screening approach can be a means for identifying women at high risk of fracture.</p> <p>Study I includes postmenopausal women without current hormone therapy (n=3363) from the Women's Health In the Lund Area (WHILA) study and investigates the association between levels of sex steroid hormones at baseline and risk of fracture during follow-up. An increased fracture risk was observed with low levels of androstenedione and androstenedione/sex hormone binding globulin (SHBG) ratio at baseline, but no effect on fracture risk was observed with oestradiol level at baseline.</p> <p>Study II includes all postmenopausal women (with and without current hormone therapy) (n=6416) in the WHILA study and compares general risk factors at baseline and risk of fracture during follow-up. Increased fracture risk was observed with use of proton pump inhibitors (PPI) at baseline and history of previous fracture. A positive family history of diabetes decreased fracture risk during follow-up.</p> <p>Study III includes middle-aged women from the Malmö Diet and Cancer study (cardiovascular cohort) (n=2927) and investigates the association between baseline levels of blood cadmium (B-Cd) and risk of fracture during follow-up. Higher levels of B-Cd at baseline did not increase the risk of incident fracture during follow-up, but was associated with increased mortality. This increase in mortality remained when adjusting for smoking status.</p> <p>Study IV explores the feasibility of using the FRAX® algorithm to identify women at increased risk of fracture as a primary screening. Women were offered the possibility via a postal questionnaire in conjunction with routine mammography screening, internet questionnaire and a postal questionnaire. In total, 43.1 % responded to the questionnaire and 37.3 % participated in the study. The postal questionnaire rendered the largest number of responses, but the largest participation rate was in the postal mammography group.</p> <p>In conclusion, having had a first fracture is a risk factor for further fracture and hence it is important both to avoid that first fracture by identifying women with risk factors for fractures, e.g., use of PPI, but also to identify women at increased fracture risk before they break a bone by using fracture risk assessment tools like the FRAX® algorithm.</p>		
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*To Magnus, Cecilia and Viktor*

*“Arriving at one goal is the starting point to another.”*

*John Dewey (1859-1952)*



# List of original studies

This thesis is based on the following original studies, referred to in the text by their Roman numerals. The studies are appended at the end of the thesis. Reprints are made with the permission from the publisher.

- I. **Moberg L**, Nilsson P M, Samsioe G, Borgfeldt C. Low androstenedione/sex hormone binding globulin ratio increases fracture risk in postmenopausal women. The Women's Health in the Lund Area study. *Maturitas*, 75 (2013) 270-275.
- II. **Moberg L**, Nilsson P M, Samsioe G, Borgfeldt C. Use of proton pump inhibitors (PPI) and history of earlier fracture are independent risk factors for fracture in postmenopausal women. The WHILA study. *Maturitas*, 78 (2014) 310-315.
- III. **Moberg L**, Nilsson P M, Samsioe G, Sallsten G, Barregard L, Engström G, Borgfeldt C. Increased blood cadmium levels do not increase fracture risk, but increased mortality in women: The Malmö Diet and Cancer Study. *Submitted, July 2016*.
- IV. **Moberg L**, Nilsson P M, Holmberg A, Samsioe G, Borgfeldt C. Primary screening for osteoporosis with the FRAX® questionnaire – Uptake rates in relation to invitation method. *In manuscript*.



# Abstract

Fracture rates are increasing as a result of an ageing population. To identify women at increased fracture risk is of the utmost importance for the prevention of an initial fracture as well as of any further fractures. The aim of the included studies is to investigate risk factors for fractures in postmenopausal women and explore whether a screening approach can be a means for identifying women at high risk of fracture.

Study I includes postmenopausal women without current hormone therapy (n=3363) from the Women's Health In the Lund Area (WHILA) study and investigates the association between levels of sex steroid hormones at baseline and risk of fracture during follow-up. An increased fracture risk was observed with low levels of androstenedione and androstenedione/sex hormone binding globulin (SHBG) ratio at baseline, but no effect on fracture risk was observed with oestradiol level at baseline.

Study II includes all postmenopausal women (with and without current hormone therapy) (n=6416) in the WHILA study and compares general risk factors at baseline and risk of fracture during follow-up. Increased fracture risk was observed with use of proton pump inhibitors (PPI) at baseline and history of previous fracture. A positive family history of diabetes decreased fracture risk during follow-up.

Study III includes middle-aged women from the Malmö Diet and Cancer study (cardiovascular cohort) (n=2927) and investigates the association between baseline levels of blood cadmium (B-Cd) and risk of fracture during follow-up. Higher levels of B-Cd at baseline did not increase the risk of incident fracture during follow-up, but was associated with increased mortality. This increase in mortality remained when adjusting for smoking status.

Study IV explores the feasibility of using the FRAX® algorithm to identify women at increased risk of fracture as a primary screening. Women were offered the possibility via a postal questionnaire in conjunction with routine mammography screening, internet questionnaire and a postal questionnaire. In total, 2 out of 5 women wanted to participate in the study. The postal questionnaire rendered the largest number of responses, but the largest participation rate was in the mammography group.

In conclusion, having had a first fracture is a risk factor for further fracture and hence it is important both to avoid that first fracture by identifying women with risk factors for fractures, e.g., use of PPI, but also to identify women at increased fracture risk before they break a bone by using fracture risk assessment tools like the FRAX® algorithm.

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# Abbreviations

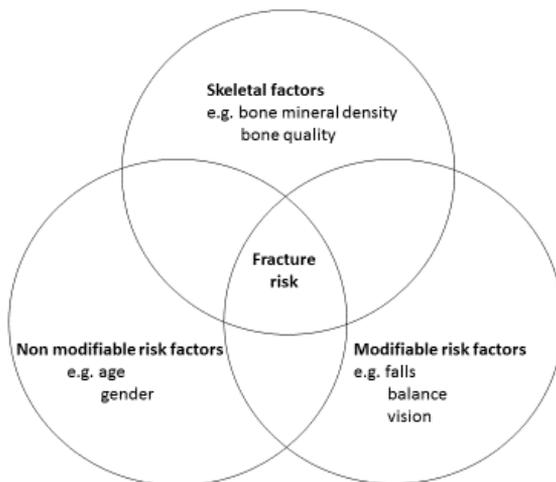
Advanced glycation end-products (AGE)  
Blood cadmium (B-Cd)  
Body mass index (BMI)  
Bone mineral content (BMC)  
Bone mineral density (BMD)  
Bone turnover markers (BTM)  
Coefficient of variation (CV)  
Combined oral contraceptives (COC)  
Confidence Interval (CI)  
Diabetes mellitus type 1 (T1D) and type 2 (T2D)  
Dual X-ray absorptiometry (DXA)  
Enzyme-linked immunosorbent assay (ELISA)  
Fracture risk assessment tool (FRAX®)  
Hip axis length (HAL)  
Hormone therapy (HT)  
Incidence rate ratio (IRR)  
International Society for Clinical Densitometry (ISCD)  
Major osteoporotic FRAX® (MO-FRAX®) score  
Magnetic resonance imaging (MRI)  
National Health and Nutrition Examination Survey (NHANES)  
Non-vertebral osteoporotic (NVOS) fracture  
Oestrogen receptor alpha (ER $\alpha$ )  
Oestrogen receptor beta (ER $\beta$ )  
Osteoblasts (OB)  
Osteoclasts (OC)  
Osteoporosis risk assessment instrument (ORAI)  
Osteoporosis self-assessment test (OST)  
Osteoprotegerin (OPG)  
Premenopausal (PM)  
Postmenopausal without hormone therapy (PMO)  
Postmenopausal with hormone therapy (PMT)  
Progestogen-only contraception (POC)  
Proton pump inhibitors (PPI)  
Quantitative computed tomography (QCT)

Receptor activator of nuclear factor- $\kappa$  B ligand (RANKL)  
Rheumatoid arthritis (RA)  
Selective serotonin reuptake inhibitors (SSRI)  
Sex hormone-binding globulin (SHBG)  
Selective oestrogen receptor modulators (SERM)  
Simple calculated osteoporosis risk estimation score (SCORE)  
Standard deviation (SD)  
Trabecular bone score (TBS)  
Ultrasound (US)  
Urinary cadmium (U-Cd)  
Women's Health Initiative (WHI) study  
World Health Organization (WHO)

# Introduction

Fracture rates are predicted to increase with an older population (1). To foresee who is going to fracture is difficult as the aetiology is multifactorial and also depends on the impact of the fall (2). Osteoporosis is a silent condition predisposing to fracture. Osteoporosis is defined by low bone mineral density (BMD) but that is only one parameter defining whether or not the result of a fall is a fracture or not. A fracture occurs when the force applied on the bone is greater than the strength of the bone.

Risk factors for fracture include skeletal factors, but also include non-skeletal factors that may affect fracture risk and influence the total fracture risk assessment. This thesis is focused on risk factors for fractures in postmenopausal women and how to identify women at increased fracture risk.



**Figure 1**  
The complex interplay between factors affecting fracture risk.



# Background

## Osteoporosis and fractures

### **Bone metabolism**

The bone consists of the mineralized matrix consisting of hydroxyapatite (calcium and phosphate), the non-mineralized matrix (e.g. collagen and metalloproteinases), and the cellular matrix (osteoblasts (OB), osteoclasts (OC) and osteocytes (OCY). The turnover of the bone can be measured by different bone turnover markers (BTM) reflecting different aspects of the matrixes of the bone, enzymes and signalling substances (3).

The skeleton is subject to a continuous turnover with the bone resorption by OCs and formation of bone by the OBs. Osteocytes are differentiated cells of OB origin and are considered to be an increasingly important regulator of bone remodelling (4). When formation and resorption are in balance the integrity of the skeleton is constant, but if an imbalance occurs, osteoporosis may develop.

### **Definition of osteoporosis**

Osteoporosis is a systemic disease characterised by low BMD and increased risk of fracture (5). In 1994, the World Health Organization (WHO) issued a consensus statement describing osteoporosis as: “A disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.” (6). With the help of a dual X-ray absorptiometry (DXA) machine, BMD can be measured to calculate T-score (comparing the values with a reference value of women aged 25 years of age) or Z-score (comparing with an age-matched reference group) and four groups can be distinguished through this (6):

Normal: A value for BMD within 1 standard deviation (SD) of the young adult reference mean.

Osteopenia: A value for BMD more than 1 SD below the young adult mean but less than 2.5 SD below this value.

Osteoporosis: A value for BMD of 2.5 SD or more below the young adult mean.

Severe osteoporosis: A value for BMD of 2.5 SD or more below the young adult mean in the presence of one or more fragility fractures.

Bone mineral content (BMC) is the amount of mineral in the measured area and BMD is calculated by division of BMC of the measured area and is hence two-dimensional and not a volumetric measurement (7). The use of T-score instead of actual raw unit measurement, evolved as a way to simplify the interpretation and comparison of BMD measurements and T-score has become a constant output from densitometry measurements (8). In order to further standardise the results of DXA measurements, it has been suggested by the International Society for Clinical Densitometry (ISCD) that for calculations of spine T-scores, manufacturers should use their own reference material, but for hip T-scores the reference database should be the National Health and Nutrition Examination Survey (NHANES) III and only if calculating Z-scores may local reference databases be used (9).

## **Other bone measurements**

The diagnosis of osteoporosis is based on the measurement of BMD with DXA, however, approximately half of the individuals that fracture do not fulfil the criteria of osteoporosis by DXA (10, 11). Thus, factors of the bone other than BMD affect fracture risk. The quality of the bone is determined by both its micro- and macroarchitecture e.g., cortical porosity, trabecular spacing, bone size and shape (12). Research is ongoing to identify other measureable parameters of the bone that can aid in the prediction of bone quality. The use of the DXA measurement in other ways than using the BMD value has been investigated, looking at, for example, different aspects of the hip geometry with, hip axis length (HAL) (12), or calculating trabecular bone score (TBS) from the lumbar spine DXA (13, 14).

It is also possible to measure the bone quality with magnetic resonance imaging (MRI), quantitative computed tomography (QCT), high resolution peripheral QCT (HRpQCT) and ultrasound (US) (15, 16). However, neither QCT nor MRI are feasible alternatives in clinical practice due to radiation levels, availability and costs (13). Routine bone biopsies to investigate the bone histomorphometry are also not realistic in a non-research setting. Interesting work using finite element calculations on the QCT measurement is also being evaluated as further help in quantifying bone quality (17). Micro-indentation is a new and interesting approach to determine bone quality relying on assessing bone properties directly at tissue level, but is currently

available only in the research situation (18, 19) and requires further validation before it becomes an aid to fracture risk assessment (20).

## Primary and secondary osteoporosis

Primary osteoporosis is due to ageing and the menopausal transition and was previously categorised as type I and type II, where type I accelerates during menopause and type II is due to ageing (21). However, this subdivision is not used anymore (22).

Secondary osteoporosis is explained by decreased BMD or an increased fragility fracture risk due to other causes than aging and the menopausal transition (23). It may be caused by several different medical conditions, and medications. The medical conditions include primary hyperparathyroidism, hyperthyroidism, growth hormone deficiency, rheumatoid arthritis, and medications known to affect bone are, e.g., glucocorticoids, heparin, antiepileptic drugs, and selective serotonin reuptake inhibitors (SSRI) (23). Other situations emerging with an increased risk of osteoporosis is after bariatric surgery due to obesity, and post-transplantation osteoporosis (23).

**Table 1**

A selection of risk factors for osteoporosis and fractures (6, 7).

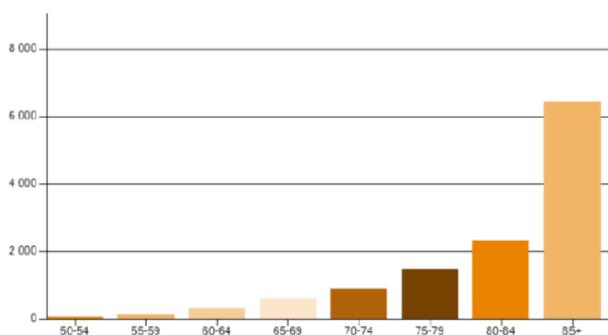
Osteoporosis	Fractures
Age	Age
Female gender	Low bone mineral density
Premature menopause	Falls
Malabsorption	Poor vision
Glucocorticoid therapy	Reduced mobility
Fragility fracture	Previous fracture
Cigarette smoking	Treatment with sedatives
Hypogonadism	
Hyperparathyroidism	
Heredity for hip fracture	
Immobilisation	
Vitamin D deficiency	
Rheumatoid arthritis	

## Risk factors for osteoporosis and fracture

There are many known risk factors for osteoporosis and for fracture. As shown in the table below, Table 1, in many instances they overlap. Some risk factors are preventable, such as, the risk of falls, but some cannot be influenced, e.g., age and gender. It is sometimes possible to refer the increased fracture risk to a certain risk factor, but in most cases it is a multifactorial process influenced by many different risk factors. As research progresses, new, previously unknown risk factors for osteoporosis and fracture are emerging, such as the use of SSRI and proton pump inhibitors (PPI) (24, 25).

The classical osteoporotic fractures are fractures of the hip, vertebrae and distal radius (2). However, it has been argued that almost all fractures occurring in an individual with osteoporosis could be due to osteoporosis, if the fall and its impact had not resulted in a fracture in an individual without osteoporosis (26). Regarding vertebral fractures, it is estimated that only one quarter to one third of the vertebral fractures are diagnosed clinically (27). Having had one vertebral fracture increases the risk of a further vertebral fracture but also increases the risk of hip and wrist fractures (28).

The annual number of osteoporotic fractures in Sweden is approximately 70 000 in both men and women and out of these, 18000 are fractures of the hip (29). The number of femoral fractures (International Statistical Classification of Diseases and Related Health Problems code S72) in Swedish women over 50 years of age in 2014, is shown in Figure 2.



**Figure 2**

The number of femoral fractures (S72) in Swedish women 2014, divided according to age (30).

## **Fracture risk assessment**

As many women who fracture do not fulfil the criteria for osteoporosis (10) work is in constant progress with identifying women at increased fracture risk regarding other aspects of bone quality as well as recognising previously unknown risk factors. In order to combine several risk factors and to estimate total fracture risk, several fracture risk assessment tools have been developed such as the WHO fracture risk assessment tool (FRAX®), Garvan, and QFracture (31-33). There are also risk assessment tools aimed at identifying low BMD, e.g., osteoporosis self-assessment test (OST), osteoporosis risk assessment instrument (ORAI) and simple calculated osteoporosis risk estimation score (SCORE) (34, 35). Some variables are included in all algorithms as shown in the Table 2. The different fracture risk assessment tools have been compared and FRAX® is currently the most studied one (36).

The various fracture and osteoporosis risk assessment tools have been compared and none of the risk assessment tools have been found to be consistently superior to the others, and some of the “simpler” tools have been found to have equal, or sometimes even better discriminative precision than some of the more complex algorithms including more variables (34, 37, 38).

Screening for osteoporosis can be performed as either primary prevention before a fracture has occurred or as a secondary screening after the occurrence of a fracture. A primary prevention strategy with a pre-screening using a fracture risk assessment tool and then a DXA measurement only in women at increased risk, was found to be a cost-effective alternative in one study (39). Secondary screening for osteoporosis in individuals with a fracture has been shown to be an effective way to decrease re-fracture rates (40).

**Table 2**

Variables included in different risk assessment tools for fracture and osteoporosis (33, 34, 37, 38).

Ofracture	FRAX*	Garvan	SCORE	ORAI	OST
Age	Age	Age	Age	Age	Age
Gender	Gender	Gender	Weight	Weight	Weight
Ethnicity	Height	Weight	Gender	Oestrogen therapy	
Smoking and alcohol status	Weight	BMD	Oestrogen therapy		
Parental history of osteoporosis or hip fracture	Previous fragility fracture	Previous fragility fracture	Previous fragility fracture		
Diabetes type 1	Heredity for hip fracture	Falls	Rheumatoid arthritis		
Care home residence	Current smoking				
Previous fragility fracture	Rheumatoid arthritis				
Dementia	Secondary osteoporosis				
Cancer	Alcohol consumption $\geq 3$ units/day				
Chronic lung disease	BMD femoral neck (optional)				
Ischaemic heart disease					
Cerebrovascular disease					
Chronic kidney or liver disease					
Parkinson's disease					
Rheumatoid arthritis					
Systemic lupus erythematosus					
Malabsorption					
Endocrine problems					
Epilepsy					
Anticonvulsants					
Antidepressants including tricyclics					
Steroids					
Oestrogen only HT					

HT - hormone therapy

BMD - bone mineral density

ORAS - osteoporosis risk assessment instrument

OST - osteoporosis self-assessment tool

SCORE - simple calculated osteoporosis risk estimation score

## The WHO fracture risk assessment tool FRAX®

The WHO Collaborating Centre at Sheffield University released the web-based FRAX® algorithm in 2008 (41). This is an algorithm that calculates the individual's 10-year probability of major osteoporotic fracture (MO-FRAX) (including hip, vertebral fracture, distal forearm and proximal humerus fracture) and hip fracture. The web-based risk score has been questioned, however, as the algorithm behind the calculations has not been made available to the public (42). Moreover certain risk factors such as number of falls are not included and neither does the algorithm take into account dose-response effects of some risk factors (43).

The use of FRAX® has been the subject of several studies and investigated how well the algorithm identifies women at increased fracture risk. The British SCOOP study used FRAX® and BMD measurements in selected cases to identify individuals at increased risk of fracture (44). Preliminary results found that the screening did not affect overall fracture risk but a decreased hip fracture risk was observed in cases compared to controls (2.6% vs. 3.5%; HR 0.73; p=0.003) (45). In the SCOOP study, FRAX® was used as a primary prevention and could show a decrease in hip fracture rates of almost 30%.

The use of FRAX with and without BMD has been investigated by *Park et al* who observed no increase in the effectiveness in identifying women with osteoporosis who later fractured by adding BMD to the FRAX® calculation (46) as was also concluded by *Kanis et al* (47, 48). A Canadian study concluded that the greatest benefit of BMD in the FRAX® algorithm was to assess individuals at an initially moderately increased risk (10-19%), whereas in the low and high risk group the use of BMD did not change handling (49). Another study found improved ability to identify high risk individuals by adding other risk factors to the FRAX® algorithm compared to using the FRAX® algorithm only (50).

The use of FRAX® in different guidelines as a cut-off for further investigation regarding fracture risk has been differently implemented in different countries. In Sweden, the National Board of Health and Welfare has set a cut-off  $\geq 15\%$  of MO-FRAX® when to consider further investigation with DXA (29). Norway and Denmark have not implemented FRAX® in their guidelines (51, 52). The US Preventive Services Task Force guideline recommends primary screening with DXA for all women aged 65 years or older and in younger women with a FRAX® risk score greater than the risk score for a white woman aged 65 years without other risk factors (risk score  $\geq 9.3\%$ ) (53).

In the United Kingdom, the National Osteoporosis Group Guidelines have implemented FRAX® to assess women aged 50 or more with a clinical risk factor, with an initial calculation of risk score without BMD and if the risk score is below an age-dependant assessment threshold then the probability of osteoporosis is considered low and no further action is planned and likewise with a risk score above the intervention threshold individuals should be considered for treatment without BMD measurement (54). If the result is above the lower assessment threshold but below the intervention threshold, then a DXA testing is recommended, followed by a new FRAX® calculation on which the decision to treat or not to treat is based (54).

## **Treatment of osteoporosis**

There are different approaches on how to treat osteoporosis. Bone formation can be increased and bone resorption decreased, both in combination with calcium and vitamin D if deficient. The effect of HT to decrease fracture risk was established with the results of the Women's Health Initiative (WHI) study (55) but due to the remaining results of the study (56), HT is not considered first line therapy for osteoporosis. The development of the selective oestrogen receptor modulators (SERM) raloxifene with agonist effect on bone, antagonist effect on breast and neutral effect on the uterus is a possible treatment (57). Current studies are also investigating the effect of the newer SERM basedoxifene with concurrent oestrogen treatment (58).

Bisphosphonates have high affinity for hydroxyapatite in the skeleton and also induce OC apoptosis (57). Treatment can be given as per oral treatment or intravenous infusions. The most commonly reported side-effects are gastrointestinal intolerance, hypocalcaemia, and myalgia and local-reactions after infusions, but the most adverse effects reported are osteonecrosis of the jaw and atypical femur fractures (57).

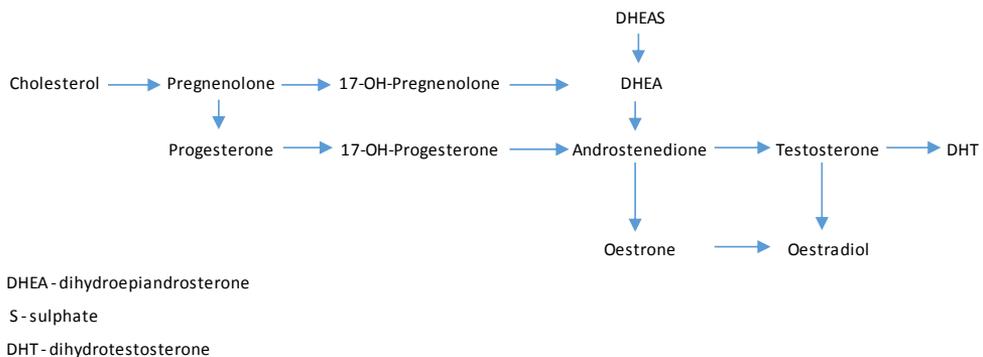
Another available treatment is with denosumab, a human monoclonal antibody binding receptor activator of nuclear factor- $\kappa$  B ligand (RANKL) inhibiting osteoclastogenesis (59). The only available anabolic treatment to date is with teriparatide, a recombinant human parathyroid hormone injected daily, stimulating both bone formation and resorption, but with an overweight for formation (57).

Research is ongoing with more treatment alternatives, e.g., romosozumab, an antibody against sclerostin, i.e. a protein that inhibits osteoblast activity and induces apoptosis via inhibition of the wingless signalling pathway; abaloparatide, a parathyroid hormone peptide analogue; and odanacatib, a Cathepsin K inhibitor that suppress bone resorption (60).

# Hormones and bone

## Sex steroid synthesis

In women, the sex steroid hormones are synthesized in the ovary, the adrenal cortex and by peripheral conversion in, for example, fat tissue (61). The sex steroid hormones consist of oestrogens, androgens and progesterone and the production is regulated by the hypothalamus in conjunction with the pituitary gland regulating the production in the ovaries and the adrenal glands.



**Figure 3**  
Schematic overview of sex steroid synthesis adapted from references (62, 63).

Ovarian production of sex steroid occurs in the theca and granulosa cells of the follicles and in the ovarian stroma from the precursor cholesterol, as shown above in figure 3. Androgens are produced in both the adrenal glands and in the ovaries. The androgens circulating in the female body, are dehydroepiandrosterone sulphate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione, testosterone and dihydrotestosterone (DHT) (62). The oestrogens available are oestradiol, oestrone and oestriol (63). The “two-cell, two-gonadotrophin” theory stipulates that luteinising hormone (LH) stimulates the production of androgens in the theca cells and when the androgens have diffused into the granulosa cells, follicle stimulating hormone (FSH) initiates the activity of aromatase converting the androgens to oestrone and oestradiol (64). Sex hormone-binding globulin (SHBG) is a carrier protein binding to oestradiol, testosterone and DHT hence regulating the levels of respective hormone that is freely available in the body (65).

The level of testosterone is not affected by menopause but declines gradually during ageing. However, oophorectomy leads to decreased levels by approximately 50% (62). Similarly, the level of DHEAS is not affected by menopause and DHEA decreases with increasing age. During the transition through menopause, the levels of oestrogen produced by the ovaries are decreased dramatically and the circulating oestrogens are predominately produced via peripheral aromatisation of androgens from the ovaries and adrenal glands (66). The production of androstenedione postmenopausally has been discussed and some studies conclude that the production from the ovary is halved from 40-50% to 20% (66, 67) whereas others find that the ovarian androgen production is negligible postmenopausally (68). *Rinaudo et al* concluded that the postmenopausal androgen production of the ovary is subject to large inter-individual variability (69).

## **Oestrogen and bone**

Oestrogen receptors are of two different types: oestrogen receptor alpha ( $ER\alpha$ ) and oestrogen receptor beta ( $ER\beta$ ) (70) with different expressions and effects on bone. Stimulation of  $ER\alpha$  leads to decreased osteoclastogenesis and increased apoptosis of OC (71) and  $ER\alpha$  is most abundant in cortical bone (70) whereas  $ER\beta$  is mostly present in trabecular bone and decreases the effect of mechanical strain on bone (70). Oestrogen receptor beta has a more suppressive effect than  $ER\alpha$  that has a generally more activating effect when stimulated by oestrogen (70).

The effect of oestrogen on bone has been studied and several different effects have been elucidated (i) increasing OC apoptosis (72, 73), (ii) stimulates osteoprotegerin (OPG) (74), and (iii) protects the cortical bone (71), and similarly oestrogen deficiency has been associated with: (i) increased apoptosis of OB and OCY (72), (ii) decreased oestrogen effect on OB progenitor cells leading to increased osteoclastogenesis (72), and (iii) increasing number of OC (70).

## **Androgens and bone**

Androgen receptors are present on both OB, OC and OCY (75-77) and androgens, for example, stimulate osteoblast proliferation, bone matrix production and synthesis of growth factors and cytokines (75). Androgens also increase the cortical thickness of the bone by both periosteal and endosteal apposition (75). Androgen insufficiency has also been associated with decreased bone mass (76). Androstenedione and testosterone are also aromatised to oestrogens in the bone which is an important source of oestrogens for the skeleton (61, 76).

## **Sex steroid hormones and fracture risk**

Levels of sex steroid hormones have been studied with regard to fracture risk. Declining levels of oestrogen have been associated with increased risk of hip (78, 79), vertebral (78), and osteoporotic fractures (80) in some studies whereas others have not observed any difference in oestrogen levels when comparing women with and without vertebral (81) or hip (82) fracture. A protective effect regarding hip fracture risk has been observed with high levels of oestrogen and oestrogen/SHBG ratio due to greater weight (83).

Low testosterone levels in women have been associated with increased hip fracture risk in women (82) but other studies have observed no effect (81, 84). Testosterone has been positively associated with increasing BMD in the lumbar spine (85) as well as showing no effect on BMD (86). Androstenedione has not been investigated in many studies but no effect on fracture risk has been observed in three studies (81, 87, 88) and one study found lower production rates of androstenedione in women with vertebral fractures but no difference in absolute levels (89).

Increasing levels of SHBG have been associated with increasing fracture risk in both the vertebrae (78, 84) and, in the hip (82, 87), as well as in osteoporotic fractures (80) in some studies but not in others (88).

## **Cadmium**

### **Cadmium background**

Cadmium (Cd) is an element occurring in the soil from both natural and industrial sources such as fertilisers and industrial emissions. Cadmium is used for the production of batteries, paint pigments, as an anticorrosive, and in the production of solar panels (90). Different Cd compounds have varying degrees of solubility in water. For example, Cd acetate and Cd chloride are quite soluble in water, whereas Cd oxide is almost insoluble in water but might be soluble at the pH level in the stomach (90). This has a practical relevance as different agricultural practices may result in an acidification of the soil and hence elevated levels of Cd in the cultivated crops (90).

Today, occupational exposure does not occur so often, the most common exposure for humans is through food and smoking. To the greatest extent Cd enters the body via ingestion or inhalation and approximately 5-10% of ingested cadmium and 10-

50% of inhaled Cd is absorbed (90). The most common source of Cd in food is through agricultural crops and in Sweden almost half of the dietary exposure is from potatoes and wheat flour (91). Cadmium is also present at higher levels in shellfish, oysters and chitterlings, for example. The mean dietary intake in Sweden is 12 µg/day and absorption is increased if there is a concomitant low dietary intake of, for example iron and calcium (90). One cigarette contains approximately 1-2 µg of Cd and on average 10% of the Cd is inhaled while smoking (90). Hence, smoking two packs of cigarettes per day for 20 years leads to an additional body burden of approximately 15 mg Cd (90).

After absorption in the lung or the gut, Cd is transported via the blood to such body tissues as the kidneys, muscles and liver and stored there with slow elimination and a half-time of decades. In the blood, Cd is to the greatest extent bound to blood cells such as erythrocytes but is also present in plasma bound to either metallothionein (MT) or to other molecular proteins. Concerning the bone tissue, less knowledge is available but it has been observed in mice studies that Cd is localized in the periosteum and bone marrow (90). Cadmium does not seem to be incorporated in the bone mineral, but may very well be present in bone cells (90).

The most commonly used biomarkers of cadmium exposure are urinary-Cd (U-Cd) and blood-Cd (B-Cd). The level of U-Cd mainly reflects long-term exposure whereas B-Cd reflects both recent and cumulative exposure (90). It has been observed in a study on biomarkers that U-Cd in smokers was associated with the duration of smoking whereas B-Cd levels were associated with the number of smoked cigarettes per day (92). The half-lives in body tissues vary between 10-40 years in the kidney and liver; and approximately 100 days for the fast component and 7-16 years for the slow component in blood (90).

## **Cadmium and bone**

Historically, the effects of Cd and bone have been observed as osteomalacia and osteoporosis in workers exposed to high levels of Cd because of their occupation and this was further substantiated in the 1950s when the Itai-Itai disease was recognised in inhabitants using water from the highly Cd-polluted Jintzu River in Japan (93, 94). The Itai-Itai disease is characterised by osteomalacia, osteoporosis, anaemia and renal tubular dysfunction (95).

The effect of chronic low level exposure to cadmium on bone has not been fully elucidated, but has been investigated more recently with regard to its effect on BMD and as a possible risk factor for fractures. The effect of Cd on bone is unknown but various theories have been suggested, such as its effect on collagen metabolism,

interference with activation of vitamin D in the kidney or with the absorption of calcium in the gut or as a direct effect on bone cells (90, 96).

The effect on BMD has been investigated in recent years and some studies have observed an impact on BMD of chronic low level exposure to cadmium as well as an increased fracture risk (97). A recent meta-analysis, observed an overall pooled relative risk of any fracture with increasing Cd levels of 1.30 (95% confidence interval (CI) 1.13-1.49), but the authors were cautious due to the heterogeneity of published studies in combination with risk of publication bias (98). A Swedish study of women found no significantly increased overall first fracture risk with increasing levels of U-Cd odds ratio (OR) 1.16 (95% CI 0.89-1.50) for all women in the study, but for never smokers the OR was 2.03 (95% CI 1.33-3.09) (99).

Osteoporosis and fracture risk has mostly been studied with regard to U-Cd and not so many studies have looked into the relationship with B-Cd. *Alfvén et al* investigated individuals over 60 years of age and found decreasing BMD with increasing B-Cd (100). *Sommer et al* did not find an increased hip fracture risk with increasing erythrocyte-Cd for both men and women, but when looking at women only there was an increased fracture risk adjusted for smoking, BMI, height and hormone replacement therapy (101).



# Aim

## Overall aim

To investigate risk factors for fractures in postmenopausal women and a possible screening approach to identify women at high risk of fracture.

## Study specific aims

### *Study I*

To delineate the association between levels of sex steroid hormones at baseline and risk of fracture during follow-up in postmenopausal women without current hormone therapy.

### *Study II*

To characterise general risk factors at baseline and risk of fracture during follow-up in women with and without current hormone therapy.

### *Study III*

To investigate the association between baseline levels of blood cadmium and risk of fracture during follow-up in middle-aged women.

### *Study IV*

To explore the feasibility of using the FRAX® algorithm to identify women at increased risk of fracture using different inclusion possibilities.



# Subjects and Methods

## Subjects

### *Studies I and II*

The Women's Health In the Lund Area (WHILA) study has been described in detail previously (102). The study was initiated in 1995 and invited all women born 1935-1945 and residing in the area round Lund on Dec 1st, 1995. All eligible women (n=10,766) were invited to participate in the study which included a postal questionnaire, physical examination, bone density measurement and laboratory analyses. The WHILA study has a participation rate of 64.2% (6917/10766).

Women were grouped according to their menopausal status: premenopausal (PM), postmenopausal without hormone therapy (HT) (PMO) and postmenopausal women with HT (PMT). In Study I, PMO women were included (n=3363), and in Study II, postmenopausal women with and without HT were included (n=6416).

Of the non-participating women in WHILA, 408 (10.6%) had been included in the study, but moved from the area before the mail invitation had been sent out (102). Non-participation has been analysed and found that during the years of 1995-1998 more non-participants than participants had died (2.6% vs. 0.2%,  $p<0.001$ ) as well as during the period of 1999-2000 (1.5% vs. 0.3%;  $p<0.001$ ) (102). The main cause of death for non-participants during 1995-1998 was cancer (n=64/99) and cardiovascular disease (n=14/99) (102).

### *Study III*

The Malmö Diet and Cancer study (MDCS) was initiated with the principal aim to investigate the relationship between diet and cancer. The study was initiated in 1991 and invited all male and female residents of Malmö born between 1923-1945 to participate in the study; this has been described in detail previously (103). Between 1992 -1994, a random selection of participants was further invited to participate in the cardiovascular cohort of the study, i.e., MDCS-CV including the baseline screening but also an ultrasonography of the carotid arteries and fasting blood sampling including B-Cd (104). In this sub-study of MDCS-CV all women

(n=2927) with available data from the baseline questionnaire, B-Cd levels and physical examination were included.

Participants (n=28098) and non-participants (n=40807) in the MDCS have been compared with regard to cancer and mortality (105). It was observed that non-participants had a non-significant decreased cancer incidence prior to recruitment relative risk (RR): 0.95 (95% CI 0.90-1.00), but an increased cancer incidence during recruitment RR: 1.08 (95% CI 1.01-1.17) compared to participants (105). Comparing mortality, non-participants had a higher mortality both during the recruitment period RR: 3.55 (3.13-4.03) and also following recruitment RR: 2.21 (95% CI 2.03-2.41) than participants (105).

#### *Study IV*

The FRAX® study was initiated in 2015 and invited a random selection of women residing in the area of Lund and born between January 1<sup>st</sup>, 1951 and August 30<sup>th</sup>, 1960 to participate in the study. The study had three different study inclusion groups all being offered the FRAX® questionnaire to fill in. The three different groups were (i) mammography, (ii) postal, and (iii) internet. Two thousand women were identified with help of the Swedish National Population Registry (“Befolkningsregistret”) and randomly offered either the postal or internet questionnaire. The mammography group included a further 1000 women who were identified via the planned screening register. A few women residing in other areas of the region who attended the local screening centre in Lund, were considered eligible participants if they fulfilled the age criteria. In total, 3000 women were invited to participate in one of the three study arms.

The FRAX® study had a response rate of 43.1 % (n=1292) and 1120 women accepted the invitation to participate in the study. A random sample of non-responders (n=20) were approached by phone and interviewed concerning to their reason for not responding to the questionnaire. The largest group of women could not remember the questionnaire (n=8). The remaining reasons were as follows: forgot to bring the questionnaire to the mammography appointment (n=2); changed the location for their mammography and hence could not return the questionnaire (n=2); did not have the time to respond (n=2); did not feel that the questionnaire was valid for her (n=2). The remaining four women were either away at the time when the questionnaire was delivered (n=1); did not want to participate (n=1); did not have the physical strength due to comorbidity (n=1); and one stated that she had returned the questionnaire but it had not been registered (n=1).

A few non-participants (n=75) had answered the questionnaire even though they did not want to participate; and they were of the same age, BMI, weight and hip-FRAX® score, but were shorter than participants (n=72; 165.0 (160.0-169.0) cm vs.

167.0 (163.0-170.0) cm;  $p=0.036$ ) and had lower MO-FRAX® scores ( $n=69$ ; 8.3 (7.2-11.0) vs. 9.4 (7.3-15.0);  $p=0.015$ ). Regarding other risk factors, fewer non-participants than participants had experienced a previous fracture (18.7% vs. 19.7%;  $p=0.001$ ), had a parent with hip fracture (6.7% vs. 20.5%;  $p=0.014$ ), or suffered from rheumatoid arthritis (4.0% vs. 4.4%;  $p=0.018$ ). More non-participants were current smokers (18.7% vs. 7.9%;  $p=0.006$ ). There was no observed difference in the number of current users of glucocorticoids (10.7% vs. 8.4%;  $p=0.53$ ), women with secondary osteoporosis (8.0% vs. 6.7%;  $p=0.18$ ), or consumers of more than 3 units of alcohol/day (0% vs. 2.1%;  $p=0.36$ ) comparing non-participants with participants.

## Methods

### Baseline data

#### *Studies I and II*

All women participating in the WHILA study were asked to fill out a questionnaire consisting of 104 questions regarding general background, medical history, medications, reproductive history and working status (102). Height and weight were measured at the primary screening. Body mass index was calculated by dividing weight (kg) with height squared ( $m^2$ ). Blood pressure (mmHg) was measured in the right arm after resting in seated position (102).

Bone mineral density measurements at the wrist were performed using a dual X-ray absorptiometry (DXA) (Osteometer DTX 200; Medi-Tech A/S, Rodovre, Denmark). A standardised phantom was used for daily calibration of the instrument and all measurements were performed by one and the same technician (102).

#### *Study III*

All participants in MDCS filled in a questionnaire at baseline inclusion in the study and were subjected to a physical examination including anthropometric variables (e.g. height, weight and blood pressure) and blood samples were drawn (106). In the MDCS-CV cohort the included subjects also had an ultrasonography of the carotid arteries and further fasting blood samples drawn.

#### *Study IV*

Women participating in the FRAX® study were asked to fill in a simple questionnaire consisting of eleven questions consistent with the FRAX® algorithm

including age, sex, height (m), weight (kg), presence of previous fracture (yes/no), parent with fracture hip (yes/no), current smoking (yes/no), current use of glucocorticoids (yes/no), rheumatoid arthritis (yes/no), secondary osteoporosis (yes/no), alcohol consumption of more than 3 units/day; and option to fill in BMD if known (41). Body mass index was calculated by dividing weight (kg) with height squared (m<sup>2</sup>) (kg/m<sup>2</sup>).

The results of the questionnaire were entered into the web-based FRAX® algorithm and individual risk scores for MO-FRAX® and hip fracture (hip-FRAX®) were calculated.

## **Fracture data**

For Studies I and II, fracture data were added from the register at the Department of Orthopaedics, Lund University Hospital and the County Council (Region Skåne). Fracture data were available from the individual inclusion in the study up until 31<sup>st</sup> of August, 2006 for study I and up until 24<sup>th</sup> May 2012 for study II. Only fractures occurring after individual inclusion in the study are included in the analysis.

In study III, fracture data were drawn, from the registries of the Swedish National Board of Health and Welfare, from individual inclusion in the study up until last update 31<sup>st</sup> of December, 2013. Only fractures occurring after individual inclusion in the study are included in the analysis. Fractures of the hip, distal radius and proximal humerus are grouped as non-vertebral osteoporotic (NVOS) fractures.

## **Laboratory analyses**

### *Study I*

Non-fasting blood samples were drawn at baseline inclusion in the study and serum aliquots were stored in a biobank until analysis for sex steroid hormones (102).

Androstenedione levels in serum were determined using an enzyme-linked immunosorbent assay (ELISA) technique (DRG Instrument GmbH, Marburg, Germany). For androstenedione, the intra- and interassay coefficients of variation (CV) were 6.3% and 8.1%, respectively. The lower detection limit for androstenedione was 0.17 nmol/L and below this a theoretical median value of 0.13 nmol/L was used (n=3).

Oestradiol was analysed using automated immunofluorescent assay (KRYPTOR®-Estradiol 17β, Brahms Ag, Heningsdorf, Germany). The intra- and interassay CVs were 7.1% and 6.0%, respectively. The lower detection limit was 3.5 pmol/L and

for oestradiol values below this a calculated theoretical mean value of 2.625 pmol/L was set (n=841).

Testosterone was analysed with an automated immune fluorescent assay (KRYPTOR®-Testosterone, Brahms Ag, Heningsdorf, Germany). The intra- and interassay CVs were 6.4% and 10%, respectively. The lower detection limit for testosterone was 0.15 nmol/L and for levels below this a set calculated theoretical mean value of 0.1125 nmol/L (n=408).

Sex hormone-binding globulin (SHBG) was analysed with ELISA technique (DRG Instrument GmbH, Marburg, Germany). The intra- and interassay CVs were 8.6% and 11.6%, respectively. For SHBG, the lower level of detection was 4.00 nmol/L and below this a calculated theoretical mean value of 3.00 nmol/L was used (n=4).

To estimate freely available levels of oestradiol and testosterone, indices were calculated as follows: [oestradiol/SHBG x 100] and [testosterone/SHBG x 100]. An androstenedione/SHBG ratio was calculated as: [androstenedione/SHBG x 100].

### *Study II*

Blood glucose was measured non-fasting and randomly during the day and analysed on capillary whole blood with a Cholestech LDX-instrument (Cholestech Corporation, Hayward, CA, USA) (107).

### *Study III*

The level of cadmium was analysed in erythrocytes and whole blood concentration was calculated using haematocrit (erythrocyte-Cd x haematocrit/100). To calculate erythrocyte concentrations, an inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California, USA) was used (108). All samples were analysed in three different rounds including two external quality control (QC) samples. The limit of detection (LOD) was 0.02 µg/L and no sample was below that limit. The results of all rounds compared to recommended limits were 0.34±0.02 µg/L (n=70) vs. 0.32-0.40 µg/L and 5.7±0.18 µg/L vs. 5.4-6.2 µg/L (108).

From the blood samples collected at baseline, C-reactive protein (CRP) was analysed by a high-sensitive method from frozen plasma with a Tina-quant CRP latex high-sensitivity assay (Roche Diagnostics, Basel, Switzerland) (109). In addition, HbA1c, insulin and whole blood glucose were analysed with standard procedures at the Department of Clinical Chemistry, Skåne University Hospital, Malmö where HbA1c was measured by ion exchange chromatography and insulin was measured by radioimmunoassay in mIU/L (110).

# Statistical analyses

## Overall statistical analysis

The Kolmogorov-Smirnov test was used to analyse distribution of data. Parametric data are presented as mean (standard deviation (SD)) and non-parametric as median (range) or (interquartile range (IQR)). Student's T-test was used for parametric data and the Mann-Whitney U test for non-parametric data. To correct for multiple comparisons, the Holm-Bonferroni test was used when appropriate. The Chi-square test was used for grouped categorical data.

All significant values were two-sided and a p-value of  $<0.05$  was considered statistically significant. Analysis was performed using the IBM Statistical Package for Social Sciences (SPSS) 18.0 (study I), 20.0 (study II), and 22.0 (study III-IV) (SPSS Inc., Chicago, IL, USA).

## Study specific statistical analysis

### *Study I*

The Pearson's correlation test was used to analyse correlation between hormone levels (logarithmic values where applicable). Cox proportional hazards regression analysis was used for analysis of hormone levels and fracture risk and to calculate hazard ratios (HR) and 95% CI.

### *Study II*

Initially, univariate crude logistic regression analysis was performed separately for all variables in Table 3 regarding general background, reproductive history, and diseases and medications. All significant statistically significant variables were then partially adjusted for age, BMI and smoking (yes/no) in a second univariate analysis.

A further multivariate fully adjusted analysis (adjusted for age, BMI and smoking (yes/no)) was then performed for all variables significant in the univariate analysis. As a sensitivity analysis, all women with prevalent fractures before inclusion in the study, were excluded in a separate multivariate analysis with identical results. To correct for missing answers, a further multivariate fully adjusted analysis was performed with imputed data.

**Table 3**

Variables analysed in univariate logistic regression in Study II.

Background factors	Reproductive factors	Diseases and medications
marital status (married vs. unmarried/widow)	age at menarche (years) (10-16 vs. <9 and > 17)	diabetes (yes/no)
education (elementary school vs. upper secondary level and university level)	duration of menstrual cycle (days) (21-36 vs. <20 and >36)	cardiovascular disease (yes/no)
current working status (full-time vs. part-time/retired)	premenopausal amenorrhea for more than 6 months (yes/no)	cancer (yes/no)
cohabiting (yes/no)	previous use of combined oral contraceptives (COC) (yes/no)	use of paracetamol (yes/no)
living in an institution (yes/no)	previous use of progestogen-only contraception (POC) (yes/no)	use of SSRI (yes/no)
number of falls/year (0 vs. 1-2 and ≥3)	parity (0 vs. 1-3 and >4)	use of PPI (yes/no)
activity during leisure time (low vs. average and intense)	age at first delivery (years) (26-34 vs. <25 and >35)	use of lithium (yes/no)
activity during work time (low vs. average and intense)	total duration of lactation (months) (0 vs. 1-18 and >18)	use of statins (yes/no)
self-reported consumed grams of alcohol per week (0 vs. 1-167 and ≥168 g)	duration of fertile period (menopause - menarche) (years) (<30 vs. >30)	use of levothyroxine (yes/no)
current smoking status (non-smoker vs. 1-14 cigarettes/day and ≥15 cigarettes/day)	age at menopause (years) (>45 vs. 40-45 and <40)	use of benzodiazepines (yes/no)
current use of "snus" (moist snuff) (no vs. yes)	HIT and/or SOEBIL (no operation vs. HIT, HIT+SOEBIL, and SOEBIL)	use of H2RA (yes/no)
body mass index (BMI) kg/m <sup>2</sup> (18.5-24.99 vs. <18.5, 25.0-29.9 and >30.0)		use of corticosteroids (yes/no)
waist-to-hip ratio (<0.85 vs. ≥0.85)		use of thiazide diuretics (yes/no)
weight gain last 5 years (>5 kg) (no vs. yes)		
weight loss last 5 year (>5 kg) (no vs. yes)		
weight gain since age 25 (kg) (<5 vs. 6-20 and ≥21)		
weight loss since age 25 (kg) (<5 vs. 6-20 and ≥21)		
self-reported fractures after the age of 40 (no vs. yes)		
family history of diabetes (no vs. yes)		
family history of fractures (no vs. yes)		
family history of cardiovascular (no vs. yes)		
systolic blood pressure (mmHg) (<140 vs. >140)		
diastolic blood pressure (mmHg) (<90 vs. >90)		
pulse pressure (systolic - diastolic blood pressure) (mmHg) (<50 vs. >50)		
non-fasting blood pressure (mmol/L) (<8.0 vs. >8.0)		
HIT - hysterectomy		
SOEBIL - bilateral salpingoophorectomy		
SSRI - selective serotonin reuptake inhibitors		
PPI - proton pump inhibitors		
H2RA - histamine type-2 receptor antagonist		

### *Study III*

Blood-Cd levels were divided into quartiles (Q) (Q1: <0.18; Q2: 0.18-0.28; Q3 0.28-0.51; and Q4: >0.51). Cox proportional hazards regression was used to calculate HR and 95% CI and were performed univariate crude, and multivariate adjusted for BMI, age, smoking status (never/previous/current smoker), self-reported diabetes mellitus, rheumatoid arthritis, gastric ulcer (verified by X-ray or gastroscopy) and asthma/chronic bronchitis. Quartile 1 was used as reference level for all analyses apart from the competing risks analysis described below.

High levels of B-Cd were associated with increasing mortality, and in order to adjust for the possible reduced relationship between B-Cd and fractures at older ages a competing risks analysis was performed by calculating sub-distribution HR and 95% CI. Quartile 4 was used as reference level. The competing risks analysis was performed using Stata Statistical Software (STATA) ver. 12.1 (StataCorp).

### *Study IV*

Between-group differences were analysed with the independent-samples Kruskal-Wallis test for non-parametric continuous data and one-way ANOVA with post-hoc Bonferroni correction for continuous data. The percentages of women with different FRAX® scores are shown with exact CI and based on the binominal distribution.

## Ethical consideration

All participants in studies I-IV participated willingly in the studies. The participation in any study may, however, affect the individual participants differently and may in some cases cause concern. A study can, for example, discover an increased risk for a disease that the participant was unaware of. The progress of research has also opened up for more elaborate analysis than originally planned for and this requires that study participants are informed.

The wish to participate in a study may also change after inclusion and it is important that all participants are aware of that they can terminate their participation in a study at any point with no impact on regular health care.

### *Studies I and II*

The original WHILA study was approved by the Regional Research Ethics Committee in Lund (LU 174-95) and for fracture collection (LU 505-03). The original WHILA study was also approved by the Swedish Data Inspection Board. All participants provided written informed consent.

### *Study III*

The original MDCS was approved by the Regional Research Ethics Committee in Lund (LU 51-90) as well as for the cadmium sub-study (2009/633). The current study was approved by the steering committee of the MDCS (2015-011). All participants provided written informed consent.

### *Study IV*

The FRAX® study was approved by the Regional Research Ethics Committee in Lund (2015/349). To access women scheduled for their routine mammography, permission was sought from and granted by the Deputy Chief Health Officer of the County Council (Region Skåne) (175-15). All women were asked to provide written informed consent and a signed returned questionnaire was interpreted as consent.



# Results

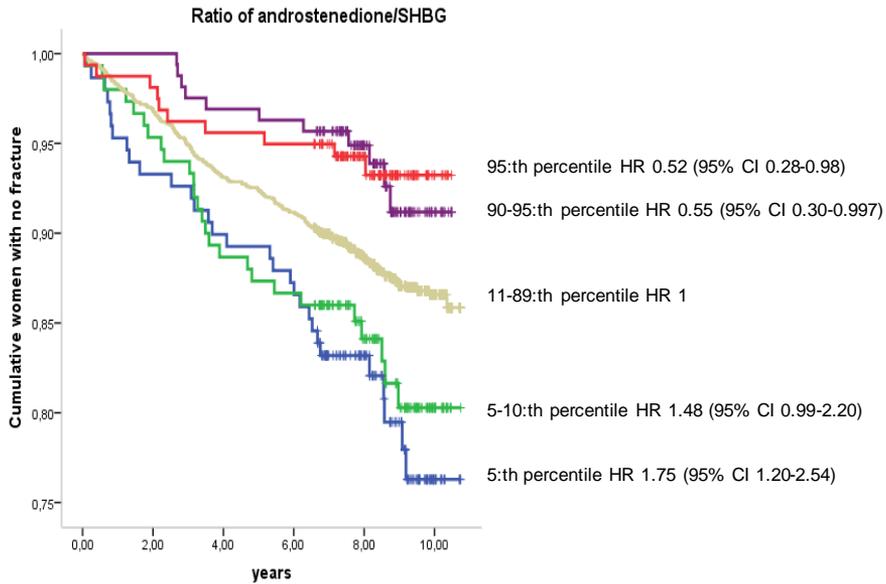
## Study I

In Study I, the PMO women (n=3363) of the WHILA study were investigated with regard to baseline levels of sex steroid hormones and fracture risk during the follow-up period. In total, 409 women had sustained at least one fracture (n=489 fractures). At baseline, women with fracture during follow-up had lower BMD ( $p<0.001$ ), T-score ( $<0.001$ ) and Z-score ( $<0.001$ ) but no observed difference in age, weight, height and BMI. Age at menopause differed significantly but the median age was the same for women with and without fracture (median (minimum-maximum)) (50.0 years (22.0-57.0)) vs. (50.0 years (25.0-60.0));  $p=0.006$ .

At baseline, women with fracture had lower levels of androstenedione ( $p<0.001$ ), testosterone ( $p=0.008$ ), androstenedione/SHBG ratio ( $p<0.001$ ), testosterone/SHBG ratio ( $p=0.003$ ), and higher levels of SHBG ( $p=0.005$ ) compared to women without fracture during follow-up. There was however, no observed difference in the level of oestradiol and oestradiol/SHBG. The ratios correlated with a correlation coefficient of 0.51 between testosterone/SHBG and androstenedione/SHBG ( $p<0.001$ ); testosterone /SHBG and oestradiol/SHBG 0.31 ( $p<0.001$ ); and androstenedione/SHBG and oestradiol/SHBG 0.27 ( $p<0.001$ ).

Univariate and multivariate Cox regression analysis for hormone levels and fracture risk found that only androstenedione and androstenedione/SHBG was associated with fracture risk: HR for androstenedione 0.45 (95% CI 0.32-0.64) (univariate) and 0.48 (95% CI 0.34-0.69) (multivariate), and HR for androstenedione/SHBG ratio 0.57 (95% CI 0.43-0.75) (univariate) and 0.57 (95% CI 0.42-0.77) (multivariate).

In order to analyse the effect of androstenedione and androstenedione/SHBG ratio on fracture risk, androstenedione and androstenedione/SHBG ratio were divided into their 5<sup>th</sup>, 10<sup>th</sup>, 11-89<sup>th</sup>, 90<sup>th</sup> and 95<sup>th</sup> percentile. Women in the lower percentiles of androstenedione and androstenedione/SHBG ratio had an increased fracture risk and women with the highest level of androstenedione/SHBG ratio had a decreased fracture risk, Figure 4. The results remained when adjusting for age, BMI and smoking status.



**Figure 4**  
Kaplan-Meier curve for androstenedione/SHBG ratio and fracture risk in Study I.

There was a positive correlation between BMD and androstenedione ( $p < 0.001$ ), testosterone ( $p = 0.001$ ), oestradiol ( $p < 0.001$ ) with the greatest correlation coefficient for androstenedione (0.11). Sex hormone binding globulin had a negative correlation coefficient of  $-0.24$  ( $p < 0.001$ ). The corresponding ratios were also significantly associated with BMD with androstenedione/SHBG with the greatest correlation coefficient of  $0.23$  ( $p < 0.001$ ).

## Study II

Study II focuses on the postmenopausal women in the WHILA study with and without hormone therapy (PMO+PMT) ( $n = 6416$ ). The baseline risk factors in the women's background factors, reproductive factors and diseases and medications were analysed with regard to fracture risk during the follow-up period.

During follow-up, 903 women sustained one or more fractures ( $n = 1137$ ). These women were of the same age and weight as those who had not sustained any fracture. Moreover the women with fracture had a lower BMI ( $p = 0.004$ ) and lower

BMD ( $p<0.001$ ), T-score ( $p<0.001$ ) and Z-score ( $p<0.001$ ) and were taller ( $p=0.005$ ). Fewer women with fracture were married (68.7% vs. 72.5%;  $p=0.019$ ). More women with fractures reported falling down 1-2 times per year (14.3% vs. 11.8%;  $p=0.017$ ) or  $\geq 3$  per year (5.3% vs. 3.4%;  $p=0.002$ ) and had sustained a previous fracture after the age of 40 years (13.5% vs. 9.1%;  $p<0.001$ ). Fewer women with fracture reported a positive family history of diabetes (7.8% vs. 10.5%;  $p=0.013$ ).

Self-reported use of medication at baseline differed in that more women with fracture used PPI (3.3% vs. 1.7%;  $p=0.001$ ), SSRI (4.8% vs. 3.1%;  $p=0.012$ ), and corticosteroids (1.4% vs. 0.7%;  $p=0.030$ ). Fewer women with fracture had used combined oral contraceptives (COC) previously (53.3% vs. 58.3%;  $p=0.009$ ). More women with fracture had at some time previously in their life, had suffered from a bout of amenorrhea for more than 6 months not associated with contraception, pregnancy or lactation (6.9% vs. 5.0%;  $p=0.019$ ).

The effect of weight on fracture risk was further investigated by analysing current BMI, weight gain or loss since the age of 25 years, and weight gain or loss during the last 5 years before inclusion in the study. Being underweight (BMI  $< 18.5$  kg/m<sup>2</sup>) when included in the study increased fracture risk during follow-up adjusting for age and current smoking (yes/no): OR 1.85 (95% CI 1.10-3.13). Being overweight (BMI  $> 30$  kg/m<sup>2</sup>) decreased fracture risk in the crude analysis: OR 0.76 (95% CI 0.59-0.99), but not when adjusted for age and current smoking (yes/no): OR 0.77 (95% CI 0.59-1.01). No effect on fracture risk was observed with weight gain or loss ( $> 5$  kg) for the last 5 years or weight loss since the age of 25 years, but having gained  $\geq 21$  kg since the age of 25 years decreased fracture risk: OR 0.73 (95% CI 0.55-0.97) when analysed crude but not when adjusting for age and current smoking (yes/no): OR 0.76 (95% CI 0.57-1.01).

In the univariate logistic regression being unmarried/widow, falling down yearly, sustaining a fracture previously, use of SSRI, PPI, corticosteroids all increased fracture risk whereas family history of diabetes and previous use of COC decreased fracture risk, Table 4. Age at menopause between 40-45 years increased fracture risk when adjusted for age, BMI and smoking status (yes/no): HR 1.36 (95% CI 1.02-1.81). In the multivariate logistic regression with original data, previous fracture: OR 1.70 (95% CI 1.24-2.32) and use of PPI: OR 2.53 (95% CI 1.28-4.99) increased fracture risk, and family history of diabetes decreased fracture risk: OR 0.66 (95% CI 0.44-0.98), see table 4. Women with a positive family history of diabetes had a slightly higher non-fasting glucose level than women without family history of diabetes ( $p<0.001$ ) but also a higher BMD ( $p<0.001$ ) and BMI ( $p<0.001$ ).

**Table 4**

Influence of general background factors, diseases, current medications and reproductive risk factors on fracture risk in univariate and multivariate logistic regression analysis (significant odds ratios (OR) 95% confidence interval (CI) in bold in Study II.

		all women		all women		all women	
		univariate crude		multivariate fully adjusted*		multivariate fully adjusted*	
		n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
marital status	married	4615	1.0	2030	1.0	4615	1.0
	unmarried/w idow	1790	<b>1.20 (1.03-1.40)</b>	741	1.08 (0.85-1.37)	1790	1.14 (0.97-1.33)
number of falls/year	0	5243	1.0	2320	1.0	5243	1.0
	1-2	781	<b>1.28 (1.04-1.57)</b>	351	1.12 (0.82-1.53)	781	1.23 (0.996-1.51)
	≥3	236	<b>1.65 (1.19-2.29)</b>	100	1.42 (0.84-2.42)	236	<b>1.57 (1.12-2.19)</b>
fractures after age of 40	no	5721	1.0	2482	1.0	5721	1.0
	yes	623	<b>1.56 (1.27-1.93)</b>	289	<b>1.70 (1.24-2.32)</b>	623	<b>1.50 (1.21-1.87)</b>
family history of diabetes	no	5382	1.0	2485	1.0	5382	1.0
	yes	649	<b>0.72 (0.56-0.93)</b>	286	<b>0.66 (0.44-0.98)</b>	649	<b>0.71 (0.54-0.92)</b>
use of SSRIs <sup>1</sup>	no	6037	1.0	2696	1.0	6037	1.0
	yes	214	<b>1.55 (1.10-2.18)</b>	75	1.57 (0.89-2.78)	214	<b>1.47 (1.04-2.09)</b>
use of PPIs <sup>2</sup>	no	6130	1.0	2724	1.0	6130	1.0
	yes	121	<b>2.03 (1.34-3.09)</b>	47	<b>2.53 (1.28-4.99)</b>	121	<b>2.01 (1.31-3.08)</b>
use of p.o. corticosteroids	no	6198	1.0	2750	1.0	6198	1.0
	yes	53	<b>1.98 (1.06-3.72)</b>	21	1.18 (0.39-3.64)	53	1.87 (0.99-3.54)
previous use of COC <sup>4</sup>	no	1718	1.0	971	1.0	1718	1.0
	yes	3695	<b>0.81 (0.69-0.95)</b>	1800	0.82 (0.66-1.03)	3695	<b>0.79 (0.67-0.93)</b>
age at menopause	>45 years	3697	1.0	2484	1.0	3697	1.0
	40-45 years	374	1.31 (0.99-1.73)	246	1.04 (0.71-1.51)	374	1.28 (0.96-1.70)
	<40 years	64	1.09 (0.55-2.15)	41	1.04 (0.45-2.43)	64	1.00 (0.50-2.00)
amenorrhoea for 6 months or more <sup>5</sup>	no	5879	1.0	2636	1.0	5879	1.0
	yes	336	<b>1.40 (1.06-1.87)</b>	135	1.37 (0.87-2.14)	336	1.34 (1.00-1.79)

\*adjusted for age, BMI and current smoking status (yes/no)

"multivariate analysis including all variables in table 3 adjusted for age, BMI and current smoking status (yes/no)

<sup>1</sup>selective serotonin reuptake inhibitors

<sup>2</sup>proton pump inhibitors

<sup>4</sup>combined oral contraceptives

<sup>5</sup>premenopausal not associated to contraception, pregnancy and lactation

In the multivariate logistic regression with imputed data, the result of the initial multivariate analysis remained with the addition that also use of SSRI at baseline: OR 1.47 (95% CI 1.04-2.09),  $\geq 3$  falls per year: OR 1.57 (95% CI 1.12-2.19) increased fracture risk and previous use of COC decreased fracture risk: OR 0.79 (95% CI 0.67-0.93).

## Study III

During follow-up for a median of 20.3 years (IQR 14.4-21.2), a total of 850 first incident fractures were observed in the 2927 women included in this sub-analysis of the MDCS-CV. They were divided into quartiles (Q) according to their level of B-Cd. There was no difference in first incident any fractures ( $p=0.83$ ) or NVOS fractures ( $p=0.37$ ) between the women in Q4 and Q1. However, more women in Q4 died during follow-up ( $p<0.001$ ) than in Q1. Women in Q4 had lower weight ( $p=0.001$ ), and BMI ( $p<0.001$ ) than those in Q1. Women in Q4 were more often unmarried ( $p<0.001$ ), less often cohabiting ( $p<0.001$ ), more often current smokers ( $p<0.001$ ) and consumed more alcohol ( $p=0.001$ ) than women in Q1.

In the univariate analysis, increased fracture risk was observed with increasing age: HR 1.05 (95% CI 1.04-1.06), self-reported treatment for diabetes mellitus: HR 1.85 (95% CI 1.22-2.81), gastric ulcer: HR 1.38 (95% CI 1.07-1.77), and asthma/chronic bronchitis: HR 1.36 (95% CI 1.05-1.76) but no effect was observed by increasing levels of B-Cd. In the multivariate analysis, the results remained with the addition that increasing BMI decreased fracture risk: HR 0.97 (95% CI 0.95-0.99).

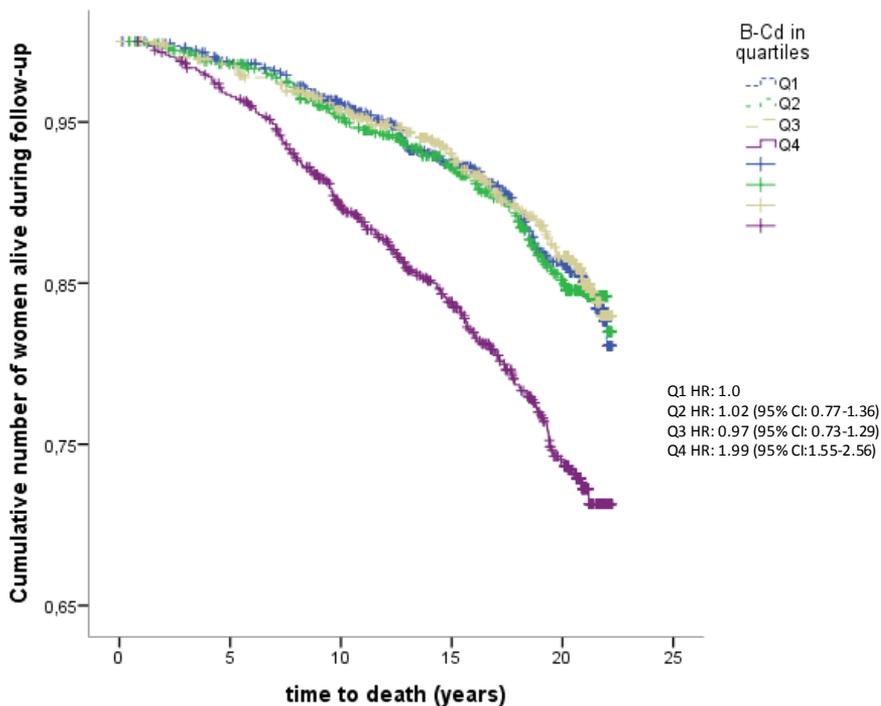
Regarding mortality, increasing levels of B-Cd were associated with increased mortality: HR 1.99 (95% CI 1.55-2.56) in the univariate analysis, and this was also increased by current smoking: HR 1.81 (95% CI 1.45-2.25), increasing BMI: HR 1.04 (95% CI 1.02-1.06), increasing age: HR 1.11 (95% CI 1.09-1.13), diabetes mellitus: HR 2.64 (95% CI 1.62-4.31), gastric ulcer: HR 1.62 (95% CI 1.17-2.25), and asthma/chronic bronchitis: HR 1.62 (95% CI 1.16-2.25). In the multivariate analysis, gastric ulcer and asthma/chronic bronchitis was no longer significantly associated with increased mortality, but increasing B-Cd, smoking status, age, BMI and treatment for diabetes mellitus remained significant.

Excluding women with prevalent fractures before inclusion in the study ( $n=78$ ), did not affect HRs for fracture risk: HR 1.09 (95% CI 0.90-1.33) nor mortality: HR 2.06 (95% CI 1.59-2.66) associated with increasing B-Cd levels. A competing risks

analysis was performed, as increasing B-Cd was associated with increased mortality and that could affect the relationship between fractures and B-Cd with increasing age. However, the fracture risk was not affected: (Q4 as HR 1.0) sub-distribution HR (SHR) for Q1 0.98 (95% CI 0.81-1.18) performed univariate, and multivariate SHR: 1.04 (95% CI 0.78-1.39).

Women currently using HT at baseline (n=500) had a decreased fracture risk during follow-up: HR 0.67 (95% CI 0.55-0.82), and also decreased mortality: HR 0.75 (95% CI 0.58-0.98). Added to the multivariate analysis did not affect the risk of B-Cd on fracture risk: HR 1.06 (95% CI 0.59-1.44) but the effect of B-Cd on mortality was attenuated: HR 1.45 (95% CI 0.96-2.18).

Kaplan-Meier curves for women in Q1-4 and mortality during follow-up are shown in Figure 5.



**Figure 5**  
Kaplan-Meier curve for blood cadmium in quartiles 1-4 and mortality, Study III.

## Study IV

A total of 1292 women responded to the questionnaires and 1120 women agreed to participate. The largest number of responses was in the postal group (n=511), but the largest participation rate was in the mammography group (92.6%).

Comparing the women participating in the different groups found that those in the mammography group weighed more (p=0.044), were slightly older (p<0.001) and had a higher BMI (p=0.004) than the women in the postal and internet groups. Fewer women in the postal group stated they were suffering from rheumatoid arthritis (RA) (p=0.004) but more suffered from secondary osteoporosis (p=0.022) than the women in the other two groups.

The median (IQR) major osteoporotic FRAX® (MO-FRAX®) score for all women was 9.4% (7.3-15.0) and the median hip-FRAX® score was 1.9% (1.2-3.2). The women in the mammography group had a higher MO-FRAX® score than both the women in the postal group (p=0.005) and those in the internet group (p=0.001), and a higher hip FRAX® score than those in the postal- (p=0.019) and internet groups (p=0.001).

The number of women with MO-FRAX® <15% was 811 (72.4%) and 298 (26.7%) had MO-FRAX® score ≥15%.

In general, the participants had few problems filling in the questionnaire either directly on the internet or on paper. In the internet group, 90.9% had filled in the questionnaire correctly, and for the mammography and postal groups only 47 answers (5.6% of the corresponding questionnaires) were changed. The most commonly changed questions were regarding use of glucocorticoids (n=17); RA (n=8); and secondary osteoporosis (n=8).



# Discussion

Identifying risk factors for fractures is of utmost importance to decrease fracture risk. Studies I-III have identified some background factors that affect fracture risk. Some are well-known such as the increased fracture risk of having had a previous fracture as observed in study II (111), whereas other findings need further elucidation.

## Hormones and bone

The findings of study I emphasise the importance of androgens in bone health in women. Women with fracture during follow-up had lower levels of both androstenedione and testosterone at baseline and low levels of androstenedione and androstenedione/SHBG were associated with increased fracture risk during the follow-up period. No difference was, however, observed regarding oestradiol levels at baseline. Regarding oestradiol there is one considerable weakness in the study. Due to limitations of the method of analysis, for 841 women with results below the lower detection level of 3.5 pmol/L, a calculated theoretical median value of 2.625 pmol/L was used for the analyses. How this has affected the results of oestradiol is unclear, but it could be argued that due to this, the effect of any differences in the hormonal levels in the lowest range could be underestimated.

Early postmenopausal women may still have some production of oestrogen from the ovaries (112) whereas further along the menopausal transition, the peripheral aromatisation from androgens is the greatest source of oestrogens (66). In this group of women in Study I, the median age at inclusion was 56.8 years and the median age at menopause was 50.0 years so for many of these women several years had elapsed since menopause both at baseline inclusion and at the occurrence of fracture.

Androgen receptors are prevalent on bone cells and *in vitro* studies suggest that androgens stimulate OB proliferation and maturation, decrease OB apoptosis, and also inhibit OC resorption on bone (113). Oestrogen deficiency is associated with increased osteoclastogenesis, decreased OC apoptosis, decreased OB proliferation and increased OB apoptosis (114, 115). Oestrogen is now more and more considered the main regulator of bone health in both men and women (115, 116) but this could not be substantiated by the results of this study.

The effect of the hormonal changes on fracture risk has been extensively studied. Increased fracture risk has in several studies been observed with lower levels of oestradiol in women (78-80, 117) and in both men and women (84). Lower levels of bioavailable oestradiol have also been associated with increased fracture risk in men (118) and women (87). Other studies have found no effect of oestradiol levels on overall fracture risk in women, but associated with increased risk of vertebral fractures (81, 117). Several studies in men have not observed any difference in oestradiol and fracture risk (119-121). High levels of SHBG have repeatedly been associated with increased fracture risk in women (78-80, 82), women and men (65, 84), and in men (118-120).

Androstenedione has previously only been investigated in four studies with no effect on fracture risk (79, 81, 87, 88). Whether the increased fracture risk observed in Study I is a direct effect of decreasing levels of available androstenedione on bone or decreased amounts of substrate available for conversion to oestrogen is unclear.

### **Diabetes mellitus and fracture risk**

Diabetes mellitus type 1 (T1D) and type 2 (T2D) have been identified as risk factors for fracture (122, 123). Study II did not observe any effect on fracture risk of diabetes itself: OR 0.97 (95% CI 0.61-1.55) and a positive family history of diabetes was associated with decreased fracture risk: OR 0.72 (95 % CI 0.56-0.93). In Study III, however, self-reported treatment of diabetes increased fracture risk: HR 1.85 (95% CI 1.22-2.81) and mortality: HR 2.64 (95 % CI 1.62-4.31).

Women with a positive family history of diabetes in Study II had a slightly higher non-fasting b-glucose level at baseline: 6.0 mmol/L (3.9-18.5) vs. 5.9 mmol/L (2.8-23.4),  $p < 0.001$ ; and also a higher BMI and BMD than women without this family history. The preventive effect of a positive family history of diabetes on fracture risk is difficult to conclusively delineate. A study from Malmö observed that hyperglycaemia was associated with decreased fracture risk (124) and hence it could be that this slight difference in b-glucose decreased osteoclastogenesis (125) and that is the reason for this finding. However, it could also be that women with positive heredity for diabetes due to lifestyle factors have increased risk of being overweight, which is shown by the increase in BMI, and it is this increased weight bearing effect on the skeleton with increasing BMD that is protective.

In these two studies it is, however, not known whether it is T1D or T2D that the women were suffering from nor for how long they have had their diagnosis. The effect of diabetes on bone has been attributed to several different factors. The bone itself is more fragile than bone in non-diabetic subjects at a given BMD and in T2D an increased cortical porosity has been observed (122). The accumulation of

advanced glycation end-products (AGE) interfering with the collagen fibres in the bone has also been described, increasing the fragility and fracture risk (126). Bone metabolism measured by BTMs has also been shown to decrease in diabetic individuals but with normal to increased levels of bone specific alkaline phosphatase (B-ALP) rendering the bone hypermineralized but decreased in quality (127). The effect of antidiabetic medication has also been described with both increased fracture risk with the use of thiazolidinediones, or neutral to protective effect on bone by metformin (128). Other factors involved in fracture risk in diabetic individuals are factors increasing the risk of falls such as hypoglycaemia, peripheral neuropathy and impaired vision (122). In Study II, increased risk of falling down  $\geq 3$  times per year was associated with increased fracture risk: OR 1.65 (95% CI 1.19-2.29).

### **Proton pump inhibitors and gastric ulcer**

In Study II, use of PPI at baseline increased fracture risk during follow-up: OR 2.03 (95% CI 1.34-3.09), and in Study III, diagnosis of gastric ulcer at baseline was associated with increased fracture risk: HR 1.38 (95% CI 1.07-1.77). It had been observed previously that surgery for gastric ulcers was associated with increased fracture risk (129). The first study regarding PPI and increased fracture risk was published by *Yang et al* in 2006 who reported that long-term use ( $>1$  year) of PPI increased hip fracture risk: adjusted OR 1.44 (1.30-1.59) (130). This study was followed by more studies regarding fracture risk that could (24, 131) and could not (132, 133) corroborate the findings. In 2011, two meta-analyses were published, substantiating the initial results of increased overall fracture risk with use of PPI: OR 1.16 (95% CI 1.04-1.30) (134) and OR 1.20 (95% CI 1.11-1.30) (135).

The effect of PPI causing increased fracture risk has been discussed and the most common conclusion is that it is the increase in pH in the stomach during treatment with PPI that decreases calcium absorption (136), but newer studies have also observed an increased risk of falls to be associated with PPI treatment (137, 138). A recent study in the field of cardiovascular disease, has observed that PPIs affected the proton pumps of the lysosomes in the endothelium and compromised the endothelial function leading to increased risk of cardiovascular and renal morbidity and mortality (139). Proton pump inhibitors have also been found to inhibit lysosomal enzymes (140). Lysosomal enzymes are important for bone cells and their signalling (141), and if PPIs interfere with the lysosomes in bone cells then that could be a possible explanation for the observed results.

The limitations of Studies II and III regarding use of PPI and gastric ulcer disease, are that neither the indication for nor the duration of treatment with PPI are known.

Moreover, it is not known how many women during follow-up have had PPI treatment or gastric ulcer disease. The results of Studies II and III further substantiate that PPI treatment and effects of gastric ulcer are associated with increased fracture risk.

## Cadmium

The results of Study III further substantiate previous findings that increasing levels of U-Cd and B-Cd are associated with increased all-cause mortality in men (142), and for men and women (143). A relatively large meta-analysis of six studies found an HR for all-cause mortality of 1.44 (95% CI 1.25-1.64) comparing the highest levels of U-Cd with the lowest levels (144). In Study III, higher levels of B-Cd, were associated with increased mortality: HR 1.99 (95% CI 1.55-2.56) in the univariate analysis and HR 1.55 (1.04-2.32) in the multivariate analysis, which is similar to the results of *Larsson et al* above (144).

Interestingly, if adjusting for use of HT at baseline in the multivariate analysis, the effect of B-Cd on mortality was no longer significant: HR 1.45 (95% CI 0.96-2.18). Use of HT was beneficial overall, decreasing both fracture risk: HR 0.67 (95% CI 0.55-0.82) and mortality: HR 0.75 (95% CI 0.58-0.98) in the univariate analysis. After the initial results of the WHI study, reporting an increased overall health risk with the use of HT (56), a further age-stratified analysis regarding mortality revealed a decreased mortality in younger women (50-59 years of age) using HT: HR 0.70 (95% CI 0.51-0.96) (145). In the guidelines, published in 2015 by the British National Institute for Health and Care Excellence, it is acknowledged that since the results of the WHI study there has been a decrease in prescription rates of HT treatment and that there is a risk that some women, suffering from menopausal symptoms refrain from using HT because of an unsubstantiated fear of adverse events (146).

Regarding the effect of cadmium on bone and fracture risk, several previous studies have observed a negative effect on BMD with increasing U-Cd levels (99, 147-150), but one study could not substantiate this (151). A review from 2013, observed an increasing risk of osteoporosis with increasing U-Cd, pooled OR 1.82 (95% CI 1.63-2.02) (152). Studies regarding fracture risk have observed both increased fracture risk in men with increasing dietary intake of cadmium (153), higher fracture risk with increasing U-Cd in men (154), increased hip fracture risk in women, but not men, with increasing B-Cd (101), and increased risk of forearm fracture in individuals >50 years of age (155). Two studies have, however, similar to the results of Study III, not observed an increased fracture risk in women regarding any first fracture: OR 1.16 (95% CI 0.89-1.50) (99) or regarding hip fracture incidence rate ratio (IRR) 0.96 (95% CI 0.92-1.01) (156).

One review concerning non-renal effects of cadmium concluded that cadmium is associated with an increased risk of osteoporosis and fracture risk, even at low level exposure (97). Another meta-analysis calculated a pooled relative risk (RR) for any fracture of 1.30 (95% CI 1.13-1.49) with increasing cadmium levels, but the authors advocated that the result should be interpreted with some caution due to risk of publication bias and study heterogeneity (98).

Study III could not substantiate any increased fracture risk with B-Cd in the highest quartile compared to the lowest quartile: OR 1.09 (95% CI 0.90-1.32). A relatively large number of participants with a long follow-up add substance to the results, but only the risk of any first incident fracture has been studied. If the effect of cadmium on bone cells has a differential effect on OCs compared to OBs with a preference for stimulating OCs, as suggested in cell culture systems (96), that could lead to predominance of the effect on trabecular bone with large resorption areas. There are also results from a sub-group of the WHILA cohort, observing that both B-Cd and U-Cd are negatively associated with oestradiol levels (157) and consequently it could be argued that women with high B-Cd could then have lower levels of oestradiol also affecting fracture risk as observed in some studies (116)

Hence, it would have been interesting to see if the results of Study III had been different if risk of first fragility fracture also had been studied and had included fragility fractures occurring after an initial incident non-fragility fracture. It may also be that B-Cd, reflecting both long-term and recent exposure (90), is less able to predict fractures with the long follow-up present in this study.

## **Screening for fracture risk**

Study IV is a primary preventive study with the aim to identify women at increased risk of fracture, to treat women at increased risk if diagnosed with osteoporosis and to follow-up fracture rates prospectively.

In Study IV, 37.3% of approached women chose to participate. During the last fifty years a general decline in participation rates in epidemiological studies has been observed (158). In this study, only two out of five women chose to participate. The reasons stated for this were mostly that the women felt healthy or were not interested in participating. No reminder was used in this study, and it could be argued that a reminder could have increased participation rates (159). It is of great concern what the effect of non-participation or non-response bias has on the results of the study and whether these may be applicable to a larger population (158). A separate analysis of non-participants of Study IV found that there was no difference in age, weight, BMI or hip-FRAX® score but non-participants did have lower MO-FRAX® score and were generally shorter than the participants. More of the non-

participants smoked, as has been observed in another study (160), but fewer of them had had a previous fracture, had parents with fractured hip or had RA themselves.

The age criterion for inclusion in Study IV (56-65 years of age) was chosen to enable the identification of women at increased risk, but with a possibility to follow up the participants and observe if the screening had an effect on fracture rates. There are few studies performed regarding primary prevention of fracture. In the SCOOP study, included women were between 70-85 years of age (44) and the two other currently running studies, with the aim to evaluate the effect of primary screening, have included women between 50-64 (161) and 65-80 (162) years of age. The SCOOP study observed a reduction in hip fractures in the study group compared to the control group (45). The further effect of Study IV will, however, need to be evaluated when the follow-up is completed with regard to fracture occurrence.

It could also be discussed if the screening should be offered in another setting such as in combination with a visit to the individual general practitioner (GP). In the current regional guidelines the responsibility for secondary screening fall on the primary care physician (163) and the focus is on secondary screening as opposed to primary screening. The Swedish national guidelines, however, advocate that all individuals with a MO-FRAX®  $\geq 15\%$  should be evaluated for osteoporosis and considered for a DXA measurement.

The implementation of a primary screening carries several considerations that require deliberation. For the individual, the diagnosis of a silent condition as osteoporosis can cause a biomedicalisation of the body and limit the individual (164). There is also the risk of false negative results missing an opportunity to treat an individual at risk (11). If osteoporosis is detected during screening, it has to be taken into consideration any risks with treatment with, for example, bisphosphonates (57). However, as a primary screening has previously been found to be cost-effective (39) together with the novel results of the SCOOP study reducing hip fractures (45), the results of currently ongoing studies (161, 162) and Study IV will give further guidance whether a primary preventive screening program should be implemented in Sweden.

# Conclusion

In conclusion, Studies I-III demonstrated that fracture risk in postmenopausal women:

- was increased in women with low levels of androstenedione and androstenedione/SHBG ratio at baseline (Study I),
- was increased by the use of PPI at baseline (Study II) and with self-reported history of gastric ulcer at baseline (Study III),
- was increased with a history of previous fracture (Study II),
- was decreased with a positive family history of diabetes (Study II),
- was not associated with higher levels of B-Cd at baseline (Study III).

The results of Study IV show that primary screening with the FRAX® algorithm is feasible with regard to filling out the questionnaire correctly, but to increase participation rate it is possible that the questionnaire needs to be offered in another setting.

It is important to remember that the studies performed were of an observational nature, they can identify associations but cannot prove causation. The prevalence of an individual risk factor at baseline was investigated in regard to the incidence of fractures during the follow-up period.

The results emphasise outcomes of previous studies that the use of PPI is associated with increased fracture risk but contradicts previous findings of increased fracture risk observed with lower oestradiol levels and higher Cd levels.



# Future perspectives

The impact of PPI on fracture risk needs to be further elucidated and preferably in a randomised controlled trial. Since PPI is a widely prescribed type of medication, and also available over the counter, a small increase in fracture risk may not affect the individual fracture risk greatly, but on a population level the effects may well be considerable. From that perspective it would be interesting to delineate prescription routines of PPI and if prescriptions are renewed without correctly re-evaluating the indication.

The access to large databases such as the WHILA study and MDCS-CV enables a unique possibility to conduct observational studies. In combination with available national registers in Sweden, it offers a chance to further investigate, for example, the effect of gestational length at birth, birthweight, reproductive history on not only fracture risk but also general health.

Regarding cadmium and fracture risk, it would be interesting to include all first fragility type fractures occurring during follow-up in Study III to observe if there is an association with B-Cd. A further possibility would be to investigate the MDCS-CV together with the sub-group of women in the WHILA study that have had Cd measured at baseline.

Further results from study IV will be very important. Firstly, to measure BMD in women identified as high risk women and secondly, to follow up if there is any effect on fracture rates of the initiated primary screening. If results indicate that it is possible to identify women at an increased fracture risk with FRAX® and that fracture rates decrease, it would be very exciting to implement the results on a larger scale in the region. A primary screening for fracture risk could have a considerable impact on the inhabitants of the region and on regional costs. If a primary screening was to be initiated in the region, then the results regarding use of PPI could be amended to the FRAX® questionnaire as a separate risk factor.



# Populärvetenskaplig sammanfattning

I takt med att befolkningen lever allt längre beräknas antalet benbrott att öka. Ett benbrott innebär lidande för den drabbade individen, men även stora kostnader för samhället med kostnader för sjukhusvård och rehabilitering samt ett bortfall på arbetsmarknaden. Benskörhet är en känd riskfaktor för benbrott, men då drygt hälften av alla benbrott sker hos individer som inte lider av detta, är det viktigt att identifiera ytterligare bidragande faktorer.

I de första tre studierna som inkluderats i denna avhandling, har ett flertal riskfaktorer undersökts och vissa av dem visade sig öka risken för benbrott.

I studie I undersöks kvinnor som passerat klimakteriet och inte hade hormonersättning. Resultatet visade att de kvinnor som vid studiens början hade låga nivåer av hormonet androstendion samt låg kvot av androstendion/könshormonbindande globulin (SHBG) hade en ökad risk för fraktur. Däremot kunde inte östrogennivån vid studiens start ses påverka risken för benbrott.

Studie II var fokuserad på allmänna riskfaktorer för benbrott hos kvinnor efter klimakteriet med eller utan hormonersättning. Resultaten av den studien visade att kvinnor som redan haft ett benbrott även hade en ökad risk för att få ytterligare fraktur. Användning av läkemedel mot magkatarr och magsår (protonpumpshämmare) innebar också en ökad risk för benbrott. Däremot kunde en minskad risk ses hos kvinnor som inte själva var drabbade av diabetes men som hade sjukdomen i släkten.

I studie III mättes kadmiumnivån i blodet hos medelålders kvinnor vid början av studien och jämfördes med förekomsten av frakturer under uppföljningstiden. Tidigare studier har visat en ökad risk för benbrott med högre nivåer av kadmium, men det kunde inte bekräftas i denna studie. Dock visades sig dödligheten vara förhöjd bland gruppen av kvinnor med de högsta värdena av kadmium jämfört med de lägsta. Den ökade risken kvarstod efter justering för rökning.

I studie IV undersöks möjligheten att använda ett riskbedömningsprogram, FRAX®, för att identifiera kvinnor med ökad risk för benbrott. Målet med studien på längre sikt är att utvärdera om det går att identifiera individer med ökad risk och förebygga benbrott innan de skett och om detta kan minska antalet frakturer på sikt. Tre tusen medelålders kvinnor har erbjudits möjligheten att svara på ett kortfattat frågeformulär och totalt ville ca 40 % av kvinnorna delta i studien. Enkätsvaren

användes för att beräkna en individuell riskpoäng för att ett benbrott skulle ske under de närmast följande 10 åren. Frågeformuläret erbjöds i samband med rutinmammografi, via post eller via internet. Mest positiva till att delta i studien var kvinnor i mammografigruppen.

För att minska antalet benbrott är det således av vikt att dels identifiera riskfaktorer, dels utveckla strategier för att identifiera kvinnor med ökad risk för frakturer innan de hunnit ådra sig dem.

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