Risk Factors for Fractures
– a link between metabolic bone disease and cardiovascular disease

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Ιθάκη

Σα βγεις στον πηγαιμό για την Ιθάκη,
να εύχεσαι να'ναι μακρύς ο δρόμος,
γεμάτος περιπέτειες, γεμάτος γνώσεις.
Τους Λαιστρυγόνας και τους Κύκλωπας,
τον θυμωμένο Ποσειδώνα μη φοβάσαι,
τέτοια στο δρόμο σου ποτέ δε θα βρεις,
αν μεν' η σκέψις σου υψηλή, αν εκλεκτή
συγκίνησις το πνεύμα και το σώμα σου αγγίζει.
Τους Λαιστρυγόνας και τους Κύκλωπας,
τον άγριο Ποσειδώνα δε θα συναντήσεις,
αν δεν τους κουβανείς μες στην ψυχή σου,
αν η ψυχή σου δεν τους στήνει εμπρός σου.
Να εύχεσαι νά'ναι μακρύς ο δρόμος.
Πολλά τα καλοκαιρινά πρωϊά να είναι
που με τι ευχαρίστησι, με τι χαρά
θα μπαίνεις σε λιμένας πρωτοειδωμένους,
να σταματήσεις σε εμπορεία φοινικικά,
και τες καλές πραγμάτειες ν' αποκτήσεις,
σεντέφια και κοράλλια, κεχριμπάρια κ'έβενους,
και ηδονικά μυρωδικά κάθε λογής,
όσο μπορείς πιο άφθονα ηδονικά μυρωδικά,
σε πόλεις Αιγυπτιακές πολλές να πας,
να μάθεις και να μάθεις απ' τους σπουδασμένους.
Πάντα στον νου σου νάχεις την Ιθάκη.
Το φθάσιμον εκεί ειν' ο προορισμός σου.
Αλλά μη βιάζεις το ταξείδι διόλου.
Καλλίτερα χρόνια πολλά να διαρκέσει
και γέρος πια ν' αράξεις στο νησί,
πλούσιος με όσα κέρδισες στο δρόμο,
μη προσδοκώντας πλούτη να σε δώσει η Ιθάκη.
Η Ιθάκη σ'έδωσε τ' ωραίο ταξείδι.
Χωρίς αυτήν δεν θάβγαινες στον δρόμο.
Άλλα δεν έχει να σε δώσει πια.
Κι αν πτωχική την βρεις, η Ιθάκη δε σε γέλασε.
Έτσι σοφός που έγινες, με τόση πείρα,
ήδη θα το κατάλαβες οι Ιθάκες τι σημαίνουν.

Κωνσταντίνος Π. Καβάφης, 1911

To Kerstin

Ithaca

When you set out on your journey to Ithaca
hope your road is a long one,
full of adventure, full of knowledge.
The Lestrygonians and the Cyclops,
the angry Poseidon – don´t be afraid of them:
You will never find such as these on your path,
if your thoughts remain lofty, if a fine emotion touches
your spirit and your body.
The Lestrygonians and the Cyclops,
the fierce Poseidon you will never encounter,
if you do not carry them within your soul,
if your soul does not set them up before you.
Pray that the road is long.
That the summer mornings are many, when,
with such pleasure, with such joy
you will enter ports seen for the first time;
stop at Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and coral, amber and ebony,
and sensual perfumes of all kinds,
as many sensual perfumes as you can;
visit many Egyptian cities,
to learn and learn from scholars.
Always keep Ithaca in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for many years;
and to anchor at the island when you are old,
rich with all you have gained on the way,
not expecting that Ithaca will offer you riches.
Ithaca has given you the beautiful voyage.
Without it you would have never set out on the road.
It has nothing more to give you.
And if you find it poor, Ithaca won't have fooled you.
Wise as you have become, with so much experience,
you must have understood what Ithacas mean.

Konstantinos P. Kavafis, 1911

In memory of my grandparents Georgia and Theodoros

Risk Factors for Fractures - a link between metabolic bone disease and cardiovascular disease

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ABSTRACT

Introduction: Fractures and cardiovascular disease (CVD) are a burden to society, as they result in high morbidity and mortality in both men and women.

Aim: The aim was to study prospectively modifiable risk factors for fractures in the general population and to possibly identify a link between metabolic bone disease and CVD.

Methods: Three population-based cohorts of both men and women were studied, with a follow-up time ranging from 13 to 30 years. Two methods of bone assessment, Quantitative Ultrasound (QUS) and Dual energy X-ray Absorptiometry (DXA), were compared during 7 years. Lifestyle factors, serum hormones and lipids, QUS and pharmacological treatment were studied in relation to future fractures, which were X-ray verified.

Results: A 30-year follow-up study of 7495 men, aged 46-56 years at baseline, showed that a high degree of physical activity during leisure time but not at work, high occupational class and high body mass index (BMI) were protective against hip fractures; whereas smoking, tall stature, age, interim stroke and dementia increased the risk. A 20-year follow-up of 1396 men and women, aged 25-64 at baseline, showed that previous fracture, smoking, coffee consumption and lower BMI each increased the risk of fracture, independently of age and sex. The gradient of risk for serum total cholesterol to predict fracture increased over time. A 13-year follow-up of 1616 men and women, aged 25-64 at baseline, showed that stroke, high age, female sex and physical inactivity during leisure time predicted fracture independently of other factors. Low QUS and use of tranquilizers predicted fracture in both genders. QUS correlated well with DXA. Secular trends were seen when men and women aged 35-64 in 1995 were compared with subjects of similar age in 2008, i.e., 13 years apart. The fracture incidence increased, with a higher proportion of vertebral fractures among postmenopausal women in 2008. Lower HRT use, lower serum oestradiol, and greater fall risk exposure due to more physical activity during leisure time in 2008, may explain the results. Serum total and free testosterone were lower in men in 2008 but the fracture incidence was unchanged. Serum total cholesterol and triglycerides were lower in men and women in 2008 compared with subjects of similar age in 1995.

Conclusions: Physical inactivity, smoking, high cholesterol and stroke were independent modifiable risk factors of fracture, indicative of a link between metabolic bone disease and CVD. Secular trends were seen in sex hormones and blood lipids in both genders, and in women, secular trends were also seen with regard to fracture type and incidence.

Keywords: Risk factors, general population, physical activity, sex hormones, cholesterol, stroke, fracture.

SVENSK SAMMANFATTNING

Bakgrund: Frakturer och hjärt-kärlsjukdom utgör ett stort problem för samhället och leder till ökad sjuklighet och död hos både män och kvinnor.

Syfte: Syftet var att prospektivt studera åtgärdbara riskfaktorer för frakturer i befolkningen och eventuellt identifiera en länk mellan benskörhet (osteoporos) och hjärt-kärlsjukdom.

Metoder: Tre befolkningsgrupper av män och kvinnor studerades med en uppföljningstid mellan 13 och 30 år. Två metoder för benmätning, hälultraljud och Dual energy X-ray Absorptiometry (DXA), jämfördes under 7 år. Livsstilsfaktorer, hormoner och blodfetter, benmassa och läkemedel studerades i relation till nya frakturer som var röntgenundersökta.

Resultat: Uppföljning under 30 år av 7495 män, som var 46-56 år vid studiestart 1970, visade att rökning, att vara lång, att bli äldre, ha haft stroke och demens ökade risken för höftfraktur. Hög fysisk aktivitet på fritid men inte under arbete, hög yrkesklass och högt kroppsmasseindex (Body Mass Index, BMI) var skyddande för höftfraktur. Uppföljning under 20 år av 1396 män och kvinnor, som var 25-64 år vid studiestart 1985, visade att tidigare fraktur, rökning, kaffe och lägre BMI ökade risken för frakturer oberoende av ålder och kön. Risken för serum kolesterol att förespå fraktur ökade med tiden. Uppföljning under 13 år av 1616 män och kvinnor, som var 25-64 år vid studiestart 1995, visade att stroke, hög ålder, kvinnligt kön och fysisk inaktivitet på fritiden ökade risken för fraktur oberoende av andra faktorer. Lugnande medicin och låg benmassa ökade risken för fraktur hos alla. Sekulära trender, d.v.s. förändringar i tiden, påvisades med fler frakturer och fr.a. kotkompressioner, hos kvinnor 2008 jämfört med kvinnor i samma ålder, 35-64 år, 1995. Mindre östrogenersättning, lägre östradiol och ökad fallrisk p.g.a. högre fysisk aktivitet 2008 kan ha bidragit. Testosteron var lägre hos män 2008 än hos män i samma ålder 1995 men hade inte påverkat frakturantalet. Kolesterol och triglycerider var lägre hos både män och kvinnor 2008 jämfört med jämnåriga män och kvinnor 1995.

Sammanfattning: Fysisk inaktivitet, rökning, högt kolesterol och stroke var oberoende åtgärdbara riskfaktorer för fraktur talande för ett samband mellan osteoporos och hjärt-kärlsjukdom. Sekulära trender påvisades med lägre könshormoner och blodfetter i befolkningen, och hos kvinnor, fler frakturer, särskilt kotkompressioner nuförtiden än under 1990-talet.

LIST OF PAPERS

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

I. Trimpou P, Landin-Wilhelmsen K, Odén A, Rosengren A, Wilhelmsen L. Male risk factors for hip fracture – a 30-year follow-up study in 7495 men. Osteoporos Int 2010;21:409-416.

II. Trimpou P, Odén A, Simonsson T, Wilhelmsen L, Landin-Wilhelmsen K. High serum cholesterol is a long term cause of osteoporotic fracture. Osteoporos Int 2010;22:1615-1620.

III. Trimpou P, Bosaeus I, Bengtsson B-Å, Landin-Wilhelmsen K. High correlation between quantitative ultrasound and DXA during 7 years of follow-up. Eur J Radiol 2010;73:360-364.

IV. Trimpou P, Lindahl A, Lindstedt G, Oleröd G, Hansson P-O, Odén A, Wilhelmsen L, Landin-Wilhelmsen K. Stroke should be regarded as a risk factor for fracture - a 13-year follow-up study in men and women. Submitted.

V. Trimpou P, Lindahl A, Lindstedt G, Oleröd G, Wilhelmsen L, Landin-Wilhelmsen, K. Secular trends in sex hormones and fractures in men and women. Submitted.

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CONTENTS

Abbreviations

BMC = Bone Mineral Content

BMD = Bone Mineral Density

BUA = Broadband Ultrasound Attenuation

CHD = Coronary Heart Disease

CVD = Cardiovascular Disease

DBP = Diastolic Blood Pressure

DXA = Dual energy X-ray Absorptiometry

HR = Hazard Ratio

HRT = Hormone Replacement Therapy

IGF-1 = Insulin Growth Factor-1

MONICA = MONItoring of trends and determinants in CArdiovascular disease

OR = Odds Ratio

PTH = Parathyroid Hormone

QUS = Quantitative Ultrasound

RR = Risk Ratio

SBP = Systolic Blood Pressure

SD = Standard Deviation

SE= Standard Error

SOS = Speed Of Sound

WHO = World Health Organisation

INTRODUCTION

Background

The skeleton plays a number of different roles. It protects sensitive inner organs like the lungs and heart. It is not only the place of haematopoetic activity, but it also plays an important endocrine role as it serves as a reservoir for minerals, mainly calcium and phosphorus. The skeleton is the target of many endocrine activities and is affected by mechanical stimuli and age.

Beyond that, the skeleton is the place for many muscles to be attached to, thus allowing us to move, walk and run. For the skeleton to play its roles efficiently, some properties are crucial. It needs to be light so that we can move easily. It has to be flexible, to prevent it from breaking easily; yet strong enough to avoid fractures. The skeleton is a living tissue, as it is sensitive to the mechanical load applied to it and because inactivity or abnormally low mechanical stress results in reduced bone mass.

The composition of bone

The skeleton can be divided into the axial (vertebrae and the pelvis) and the appendicular skeleton (long bones). Furthermore, bone is divided into two types; cortical bone and cancellous bone. The cortical bone is densely compacted tissue, and forms the outer layer of mainly long bones. The cancellous, or trabecular, bone is characterised by a network of trabeculae resulting in a large surface area, which makes this bone more sensitive to metabolic changes.

Bone as tissue is composed of intercellular calcified material, the bone matrix, and bone cells. The matrix, organic (~20%) and inorganic (~70%), is formed and maintained by the osteoblasts. The unique combination of organic and inorganic material results in the characteristic properties of bone; its hardness and elasticity (1-3).

Why do we fracture?

The development of a fracture is dependent on bone strength and the load applied to it by trauma.

Bone strength is determined by the properties of the bone material and bone geometry. The degree of mineralisation, the collagen characteristics and microdamage affect the bone material, whereas bone geometry is determined by the bone size and shape (bone mass) and bone morphology (distribution of bone mass and microarchitecture). Thus, whether a fracture is to be sustained depends on the load applied to the bone and the consequent strain in relation to the bone strength (1, 3, 4).

The mechanical behaviour of bone is constantly influenced by mechanical and hormonal stimulation, which affect the bone turnover and remodeling (5). Bone modelling is the process that results in bone formation alone without prior bone resorption. It occurs mainly in the growing skeleton and during fracture repair (5). Bone remodelling is the continuous process throughout life of destruction and formation of bone that replaces old bone by new bone.

The main purpose of bone remodelling is to maintain the metabolic and structural properties of the bone (5). It has been estimated that remodelling results in 5-10% of the skeleton being renewed every year and the remodelling activity is 5-10 times higher in trabecular bone than in cortical bone. Thus, bone has the unique property of self-renewal and repair (6).

However, many age-related changes occur in the skeleton, resulting in decreasing bone mass, cortical and trabecular thinning and increasing cortical porosity and trabecular perforation, as shown in Fig. 1 (7).
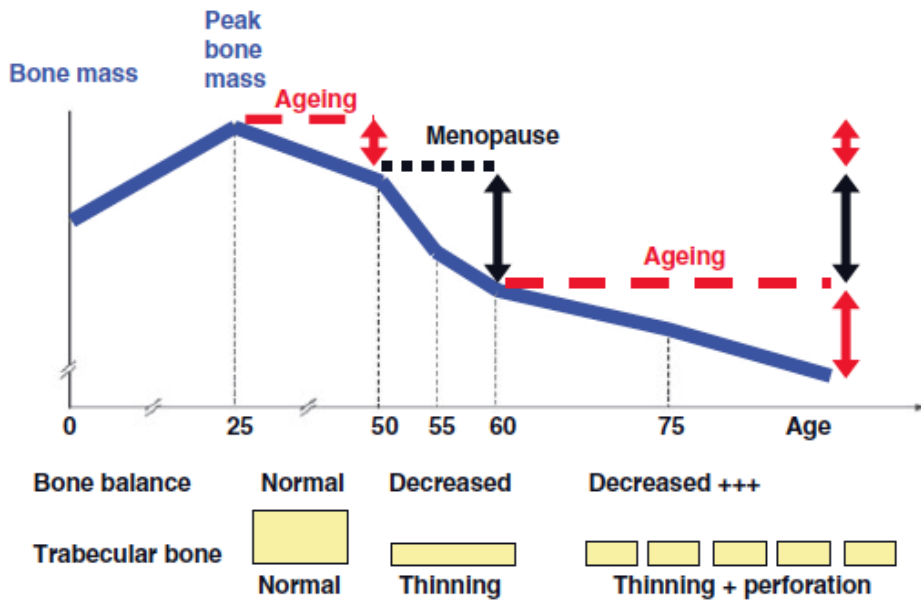
Fig. 1 The process of age-related trabecular thinning. Reproduced from (7) with permission from Oxford University Press.

Thus, older bone has poorer performance, due to decreased strength and accumulated microdamage (8-12).

Trauma is of major importance for the development of bone fracture. Risk of falling, as well as the type of trauma, high or low-energy trauma, determine the development of bone fractures (8).

What is an osteoporotic fracture?

An osteoporotic, or fragility fracture, is defined as bone failure following trauma that would otherwise not occur in a healthy skeleton, but is also described as a low energy fracture, often after a fall from a standing height (13). However, age is a major risk factor for fracture. The general approach in defining fracture sites as osteoporotic comprises the association of fractures with low bone mass as well as

with increasing fracture incidence above the age of 50 years (14-16).

The most common osteoporotic fractures are vertebral fractures, hip fractures, Colle's fracture of the forearm and proximal humerus fractures (17). Nevertheless, there are a number of other fracture sites that fulfil the above criteria and, thus, should be regarded as being of osteoporotic origin. These sites are the clavicle, pelvis, lower leg in women, wrist, heel, rib and elbow (14, 18, 19).
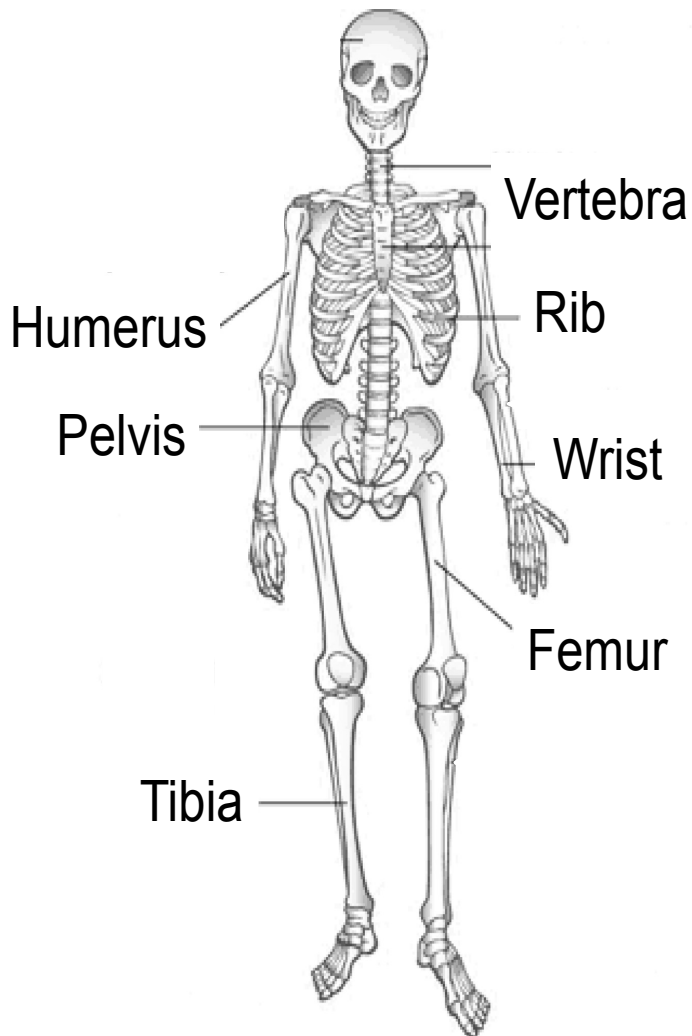

Fig. 2 The most common osteoporotic fractures.

Why are fractures important?

Osteoporotic fractures are a major burden to society worldwide in different respects. They have a huge impact on an individual's morbidity and place a progressively larger burden on health care resources (20). A study by Kanis et al. (21) showed that the estimated number of osteoporotic fractures in Europe in 2000 was 3.79 million. Of these, 0.89 million were hip fractures (179 000 hip fractures in men and 711 000 in women). The total direct costs were estimated at 31.7 billion euros, which were expected to increase to 76.7 billion euros in 2050, based on the expected changes in the demography of Europe (21).

Johnell et al. (22) showed that the number of days in hospital due to osteoporotic fracture in Sweden in 1996 was between that of ischaemic heart disease and stroke. Patients with hip fractures experience impaired quality of life and subsequent increased morbidity and cost (23). The majority of osteoporotic fractures occur in elderly women, although the prevalence of all fractures is the same for men and women over the entire life span (24).

In an attempt to calculate the global burden of osteoporotic fractures in terms of Disability Adjusted Life Years (DALY) lost because of a fracture, it was estimated that osteoporotic fractures accounted for more DALYs lost in Europe than common cancers, with the exception of lung cancer (25). The remaining lifetime risk of any major osteoporotic fracture at the age of 50 was estimated to be 46% for women and 22% for men in Sweden (26). The corresponding lifetime risk of a hip fracture was estimated at 23% for women and 11% for men (26). Women with a prior fracture have an 86% risk of a subsequent fracture (27). One in five postmenopausal women with a prior vertebral fracture will have another vertebral fracture within one year (28). Women with a prior wrist fracture run a 50% higher risk of sustaining a hip fracture (29). Thus, the fracture risk is undoubtedly increased after a first osteoporotic fracture (30).

Morbidity and fractures

Already during hospitalisation acute complications may occur, such as pneumonia, deep vein thrombosis and urinary tract infection. Co-morbidities often follow a hip fracture event. Co-morbidities are significant and the risk of death after a hip fracture increases with the morbidity score (31-33). It has been estimated that 50% of individuals able to walk before the fracture are unable post-fracture and that age is of crucial importance (32, 34). A study by Chrischilles et al. showed that 55% of patients aged over 90 with a hip fracture are discharged to nursing homes (35).

The incidence of vertebral fractures is less well documented, mainly because of the uncertainty regarding the definition of vertebral fractures. Nor is co-morbidity due to vertebral fractures well assessed, as these fractures may remain undiagnosed for a long time and whether back pain is really due to vertebral fractures is often unclear. It is estimated that of all incident vertebral fractures, 40% receive clinical attention and 10% result in hospitalisation. The major clinical disorders are back pain, kyphosis, height loss and decreased quality of life scores with increasing numbers of sustained vertebral fractures (36). Distal forearm fractures have not been shown to result in major disability, although over 50% report poor function six months afterwards (35).

Mortality and fractures

Mortality patterns have been studied for the most frequent fragility fractures, i.e., hip, vertebral, and wrist fractures (20, 23, 37-39). The mortality after a hip fracture is generally higher in men than in women, and higher for those with poor health status before the fracture, and increases with age (32, 33, 37, 40, 41). Approximately 20% of women and 30% of men die within one year after a hip fracture (20). It is estimated that the risk of mortality is 9% in men and 4% in women during hospitalisation after a hip fracture (42). The mortality risk is at its highest immediately after the hip fracture and decreases over time, so that after two

years' survival, the risk is comparable to that of the age and sex-matched general population (43). A recent study though, showed that mortality is increased 5 years following any fracture (44). Co-morbidities contribute to decreased life expectancy. The mortality excess due to hip fracture *per se* is estimated at 10-20% and approximately 50% of those surviving will also suffer long-term disabilities (23).

In the case of vertebral fractures, mortality is increased beyond a year afterwards and it is shown that mortality increases with increasing time after the fracture (45). It has been shown by van Staa et al. that twelve-month survival after a vertebral fracture was 86% versus 94% expected (46). No increased mortality has been shown for wrist fractures (37, 46).
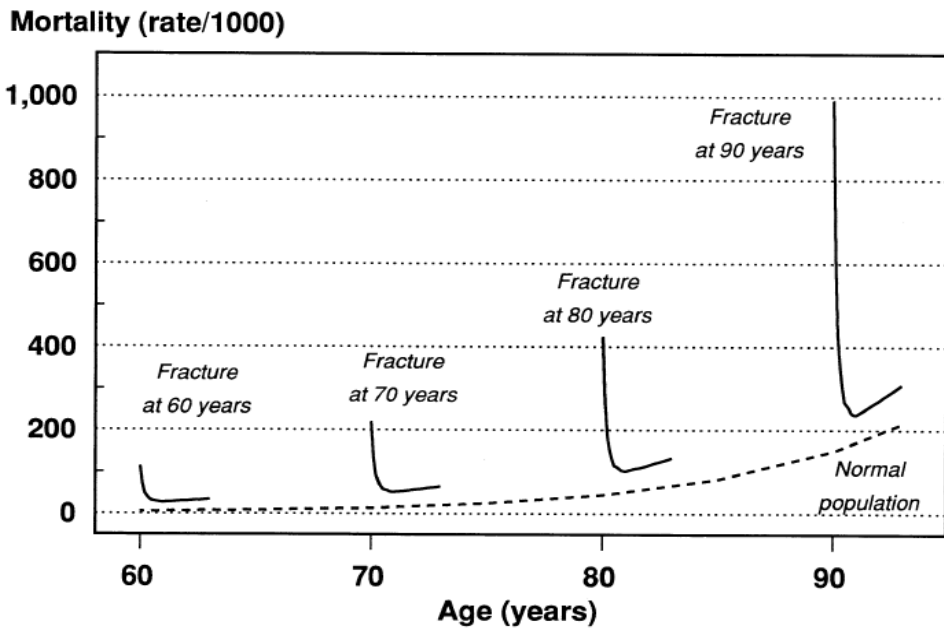

Fig. 3 Pattern of mortality in the general population and following hip fractures at the ages shown. Reprinted from (23) with permission from the Elsevier Publishing Group.

Fracture epidemiology in the world

In 2000, an estimated 8.9 million osteoporotic fractures occurred worldwide, 1.6 million of which were hip fractures, 1.7 million forearm fractures, and 1.4 million were clinical vertebral fractures. The largest number occurred in Europe (35%), where 890 000 men and women sustained a hip fracture (21, 25).

In the US, the corresponding figures are expected to increase by about 50% by the year 2025 (47). In England, it was estimated that 53% of women and 21% of men around 50 years of age will sustain a fragility fracture during their remaining lifetime (46).

The probability that a fracture will occur differs greatly, depending on the region of the world (48). Hip fracture rates vary between countries, with the highest rates in northern Europe and the lowest in Mediterranean countries, and with rates higher among women than men (49).

The prevalence of vertebral deformities across Europe was estimated at 12% for both men and women aged 50-79 years of age. In the ages between 50 and 60, the prevalence is similar in both sexes but after the age of 60 the prevalence is higher in women. Most of the vertebral fractures in older women occur during regular activities rather than as a result of falling (50). The incidence of radiographically defined vertebral fractures in Europe was estimated at 13.6 per 1000 person years for men and 29.3 per 1000 person-years in women for the age group 75-79 years, according to the European Prospective Osteoporosis Study (51).

Fracture epidemiology in Sweden

Women in Sweden have the highest incidence of hip fracture globally and the highest incidence of vertebral fracture in Europe (49, 50, 52). The lifetime risk of an osteoporotic fracture is approximately 50% for a Swedish woman and 25% for a Swedish man (26). Up to 70 000 osteoporosis-associated fractures occur every year, 18 000 of which are hip fractures, and the costs amount to some SEK 4.6 billion

per year (53-55). The exact reason is unknown but tall stature (56) with little exposure to sunlight due to the geographical latitude (57), with consequent low vitamin D levels in the general population (58), have been proposed.

What is a risk factor?

The term risk factor was first introduced in the literature in 1961 by the director of the Framingham Heart Study, Dr. Thomas Dawber, who associated specific conditions with coronary heart disease (CHD) (59, 60).

A risk factor can be defined as a variable that may influence the risk of a disease or an outcome; either increase or decrease the probability of a condition occurring/happening. The term "risk factor" is often used for factors associated with an increased probability of an individual suffering the outcome in question. A risk factor for a certain condition is evaluated by comparing the risk of those exposed to the risk factor in question to the risk of those not exposed. Risk factors do not always reveal causation but rather correlation. Typically, smoking and high cholesterol levels have been shown to be risk factors for myocardial infarction and hypertension for stroke (61-65).

Risk factors for Cardiovascular Disease (CVD)

Cardiovascular Disease as a term comprises large-vessel disorders, mainly resulting from atherosclerosis. CVD includes coronary heart disease (CHD), stroke and peripheral vascular disease. CVD is the most common cause of death in Sweden in both genders (66). Cardiovascular risk factors for CHD are smoking, high cholesterol and fibrinogen levels, hypertension, diabetes, psychosocial factors and abdominal obesity. Regular consumption of fruit, vegetables and alcohol and regular physical activity lower the risk (63). Similar risk factors were shown largely to account for the risk of stroke (65).

Risk factors for osteoporotic fractures

Several risk factors have been recognised as being associated with an increased risk of fragility fractures (Table 1). They can be divided into modifiable and non-modifiable risk factors.

Table 1 Risk factors for fragility fractures. Reprinted from (67) with permission from The Lancet Publishing Group.

Female gender	Glucocorticoid therapy*
Premature menopause	High bone turnover*
Age*	Family history of hip fracture*
Primary or secondary amenorrhoea	Poor vision*
Primary and secondary hypogonadism in men	Low body weight*
Asian or white ethnic origin	Neuromuscular disorders*
Previous fragility fracture*	Cigarette smoking*
Low bone mineral density	Excessive alcohol consumption
Long-term immobilisation	Low dietary calcium intake
	Vitamin D deficiency

*Characteristics that capture aspects of fracture risk over and above that provided by bone mineral density.

Low attenuation of ultrasound also predicts fracture (68, 69). In addition to these risk factors, rheumatoid arthritis (70, 71), inflammatory gut conditions (72, 73), hyperthyroidism and over-substituted hypothyroidism (74, 75), and primary hyperparathyroidism (76) may cause secondary osteoporosis and, subsequently, fractures.

Osteoporosis - Definition

"From the Greek, meaning porous bone"

Due to fractures, osteoporosis gained attention and acquired a diagnosis code. In 1994, the World Health Organization (WHO) defined osteoporosis as "a systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture" (17, 77).
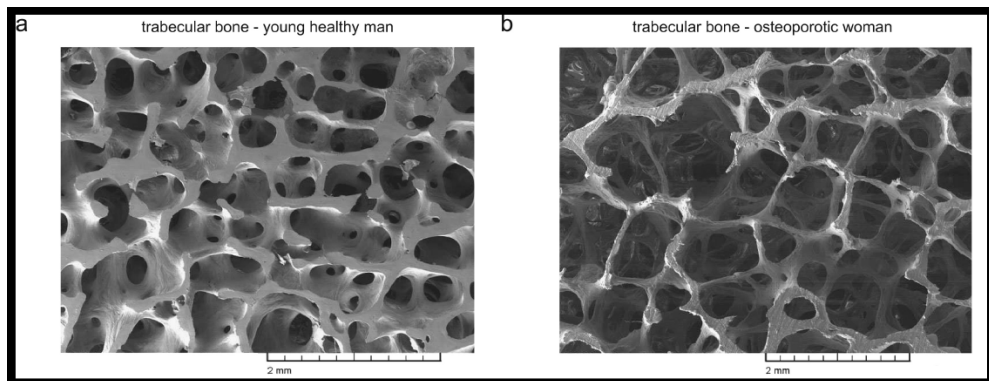
Fig. 4 Images of healthy and osteoporotic trabecular bone are shown in (a) and (b), respectively. Reprinted from (78) with permission from the Elsevier Publishing Group

Bone mass is assessed by quantitative measurement of the BMD of the femoral neck or spine. Originally, however, the diagnosis of osteoporosis was based on the BMD of the hip, spine, or forearm. BMD is expressed as the Z- or T-score.

The Z-score describes the number of SDs by which the BMD in an individual differs from the mean value expected for individuals of the same age and sex.

Z-score = [measured BMD - Age-matched mean BMD] / Age-matched SD

The T-score describes the number of SDs by which the BMD in an individual differs from the mean value expected in young healthy individuals.

T-score = [measured BMD – Young Healthy Adult mean BMD] / Young Healthy Adult SD

The reference population comprises the National Health And Nutritional Examination Study (NHANES III) reference database for femoral neck BMD measurements in women aged 20-29 years (79, 80). Based on the T-score the following thresholds of BMD are used worldwide for the definition of osteoporosis in clinical practice:

Osteoporosis - Operational Definition

Normal BMD	**T-score > -1 SD** BMD value greater than 1 SD below the young adult reference mean value.
Osteopenia or, low bone mass	**T-score <-1 SD and >-2.5 SDs** BMD value between 1 SD and 2.5 SDs below the young adult reference mean value.
Osteoporosis	**T-score ≤ -2.5 SDs** BMD value 2.5 SDs, or more, below the young adult reference mean value.
Established osteoporosis	**T-score ≤ -2.5 SDs and a fracture** BMD value 2.5 SDs, or more, below the young adult reference mean value, in the presence of one, or more fragility fractures.

In 2008, the operational definition of osteoporosis was revised; the femoral neck BMD was proposed as the standard measurement site, and the mean and SD values in young women from NHANES III were adopted as the reference population for both men and women (81).

Osteoporosis is a "silent disease" until a fracture occurs, which, accordingly, explains the clinical significance of osteoporosis. The analogy between

osteoporosis and its complication, fracture, is widely compared with other diseases, such as hypertension or diabetes, the diagnoses of which are also based on a threshold of blood pressure, or blood sugar measurement, resulting in a high risk of developing stroke or vascular complications, respectively. Bone mass assessment by determining BMD has a predictive value with respect to upcoming fractures. Marshall and colleagues showed that the age-adjusted risk of fracture at different sites increases by a factor of 1.5 to 3.0 for each SD decrease in BMD (Table 2) (82).

Table 2 Relative risk of fracture for one SD decrease in BMD below age-adjusted mean (82). Reproduced with permission from The BMJ Publishing Group.

Site of measurement	Forearm fracture	Hip fracture	Vertebral fracture	Any fracture
Measurement by methods other than QUS				
Distal radius	1.7	1.8	1.7	1.4
Femoral neck	1.4	2.6	1.8	1.6
Lumbar spine	1.5	1.6	2.3	1.5
Measurement by QUS				
Calcaneus		2.2	1.8	1.5

The ability of BMD to predict fracture is better than cholesterol's ability to predict myocardial infarction and comparable to the ability of blood pressure to predict stroke (82).

Nevertheless, the development of a fragility fracture is undoubtedly multifactorial and depends upon both skeletal and non-skeletal factors. It is also of great importance to distinguish between the assessment of BMD for the diagnosis of osteoporosis and the fracture risk assessment.

A normal BMD value; i.e., no osteoporosis according to the operational definition, does not equal no fracture risk. The risk exists but it is smaller (67). Use of BMD alone has a rather high specificity but rather low sensitivity, meaning that many individuals who will fracture in their lifetime would not be identified as being at high risk based on their BMD assessment (17, 83). Only 45% of women with osteoporosis at the age of 50 will sustain a fracture of the hip, spine, forearm or proximal humerus within the next 10 years. The vast majority of these types of fracture would occur in women without osteoporosis (16, 84-86).
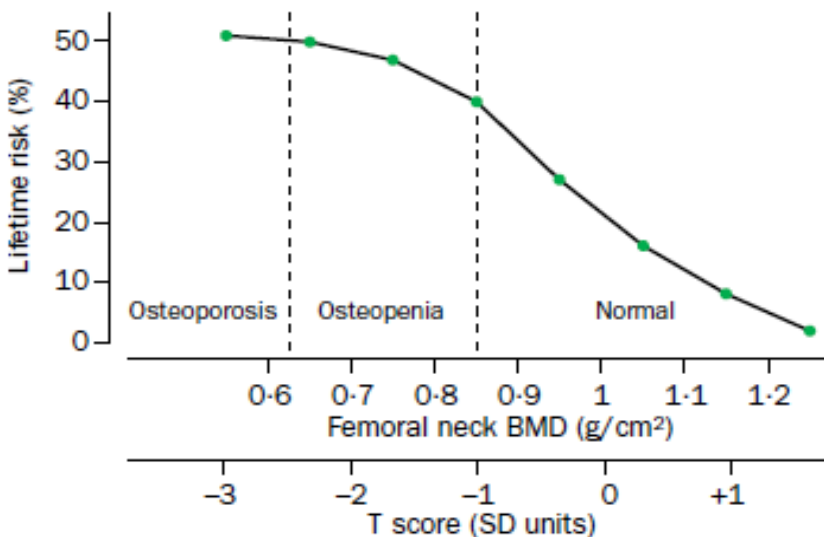

Fig. 5 The remaining lifetime risk of hip fracture in women aged 50 years according to BMD measurement or T-score at the hip. Reprinted from (67) with permission from The Lancet Publishing Group.

Thus, despite osteoporosis being a major determinant of the fracture risk, it is of crucial importance to study other modifiable risk factors and adopt population-based strategies for primary prevention of fractures. In this context, it is of importance to identify whether individuals with a high risk of fragility fractures share common risk factors with other diseases with a major impact on the health service globally, such as cardiovascular diseases.

DIAGNOSTIC ISSUES OF OSTEOPOROSIS

Several methods have been developed in an attempt to evaluate bone features both with accuracy and high precision. Almost 20 years ago, methods to measure bone density with X-ray-based techniques became available; first single X-ray absorptiometry and later the dual-energy X-ray absorptiometry, or what is internationally referred to as DXA. DXA has the advantage of reduced radiation compared with gamma-ray methods, which were used earlier. Additionally, DXA measurements are closer to the calcium content of the bone (ash weight) and have higher reproducibility rates. Thus, both the accuracy and the precision were improved. Other techniques were also developed, such as the use of ultrasound, computer and magnetic resolution tomography, as well as laser technologies.

Dual energy X-ray Absorptiometry (DXA)

DXA is based on the transmission of X-rays with high and low-energy photons through the body. The technique involves measuring the absorption of the two different energies, thus measuring two tissue components; bone and soft tissue. It is, however, assumed that the relationship between lean soft tissue and adipose tissue is constant. This may lead to measurement errors, with an impact on accuracy as well as precision (87-90). The DXA measurements assess the areal bone mineral density (aBMD, g/cm^2), and the bone mineral content (BMC, g) at the region of interest. However, DXA is a projectional technique; a three-dimensional object is described in terms of two dimensions, and it is thus impossible to assess density volumetrically (g/cm^3). This is of major importance when interpreting DXA measurements obtained from bones of different size (89, 90). Additionally, vertebral fractures, scoliosis, or even aortic calcification may result in spuriously elevated BMD values (91, 92). DXA is referred to as the "gold standard" for assessing bone mass density in clinical practice and the current definition of osteoporosis is based upon this technique.

Quantitative ultrasound (QUS)

In the QUS technique, broadband ultrasound attenuation (BUA, dB/MHZ) and the speed of sound (SOS, m/s) are used to reflect properties of bone related to density and architecture. QUS parameters are found to be associated both with BMD and with bone structure irrespective of BMD, with BUA, in particular, reflecting structural parameters (93-96). The major advantages of this technique is the fact that it is non-ionising, cheap and portable. However, the precision (reproducibility) of QUS is poorer than that of DXA, resulting in longer follow-up times between measurements than DXA (two to three-fold) to detect change (97).

Nevertheless, in adults, QUS at the calcaneus can predict fracture risk independent of DXA (98), is associated with fracture history (99), and can discriminate between cases with vertebral and non-vertebral fractures and controls (100).

Quantitative computed tomography (QCT) and peripheral QCT (pQCT)

QCT and pQCT have the advantage of analysing separately cortical and trabecular bone, bone geometry and volumetric bone density. Nevertheless, a major disadvantage is the high radiation dose. Neither of these methods has been shown to be better than DXA in predicting fragility fractures (101).

Magnetic Resonance Imaging (MRI)

Skeletal assessment is based on different amounts of water and lipids in different types of tissue. In this way, it is possible to differentiate between various anatomical structures and evaluate bone density volumetrically without radiation. MRI has so far only been used in research and its applicability in clinical practice has not been evaluated.

GENERAL AIM OF THIS THESIS

The main objective of this thesis was to examine to what extent factors measured at baseline could predict osteoporotic fractures in the long term in men and women in the general population. Furthermore, the intention was to study whether there are grounds to support the hypothesis of a possible link between metabolic bone disease and CVD. This was mainly studied with regard to known common risk factors for the two diseases but also by studying the fracture outcome following hard CVD outcome.

Specific aims

Paper I

The aim of this study was to evaluate lifestyle factors, especially physical activity at work and leisure, comorbidity, as well as other potential risk factors for hip fractures in men. We used data from a population sample of 7495 men (The Gothenburg Primary Preventive Study) aged 46-56 years at baseline in 1970, and followed the subjects for more than 30 years (203 051 person-years).

Paper II

The aim was to explore links between metabolic bone disease and CVD with access to fracture risk factors in a younger cohort. A random sample of men and women (n=1396), aged 25-64 years at baseline (The Gothenburg WHO MONICA study 1985), was followed-up for 22 years.

Paper III

The aim was to study whether changes in calcaneal QUS were correlated with changes measured with DXA and to validate prospectively calcaneal QUS against DXA. Postmenopausal women (n= 80, aged 53-73 at baseline) were followed up during 7 years and underwent repeated QUS and DXA measurements.

Paper IV

The aim was to study prospectively risk factors for osteoporotic fractures during a long follow-up period and whether CVD increased the risk of osteoporotic fracture. A random population sample of men and women (n=1616), aged 25-64 at baseline (The Gothenburg WHO MONICA study 1995), was followed up for 13 years.

Paper V

The aim was to examine possible secular trends in sex hormones and fractures. A random population was studied twice, and men and women of similar age were compared 13 years apart (The Gothenburg WHO MONICA study 1995 and 2008).

SUBJECTS AND METHODS

Ethical Considerations

All studies in this thesis were conducted according to the Declaration of Helsinki and were approved by the Ethics Committee at the University of Gothenburg. All subjects gave their verbal (*Paper I*), or written informed consent (*Papers II - V*). Furthermore, individuals with abnormal findings in blood pressure and blood samples were taken care of by the study team.

Study populations

The Gothenburg Primary Prevention Study

The Multifactorial Primary Prevention Study started in Gothenburg in 1970 and was originally an intervention trial aimed at reducing coronary events by actions against smoking, hypercholesterolaemia and hypertension in an intervention group comprising 10 000 men, a random third of all men in the city who were born between 1915 and 1925 (except those born in 1923). Initial screening was performed between 1970 and 1973 with 7495 participants, and a second screening round was carried out between 1974 and 1977 (Fig. 6). After 10 years of follow-up, 20% of the sub-samples of the intervention group and the control group were re-examined. No significant difference in risk factors or in CVD outcome between the intervention and control groups was detected. Thus, any changes brought about by intervention also happened among the general population; therefore, the present study group is considered to be representative of the population in the city.

All participants were followed from the date of their baseline examination until December 31, 2003, using their unique personal identity number (203 051 person - years). A computer file of the study cohort was run against the Swedish national register on causes of death and the Swedish hospital discharge register. The follow-up time was 30 years.

The Gothenburg WHO MONICA study 1985

In 1985, 1000 men and 1000 women of Caucasian origin, aged between 25 and 64 years, were selected at random from the population census of the city and invited to participate in the WHO MONItoring of trends and determinants in CArdiovascular disease (WHO MONICA) project. The participation rates varied from 65% among men aged 25-34, to 74% among men aged 55-64, and were the same for women in these age groups. In total, 1396 subjects (53% of whom were women), aged 25-64 years, participated in the baseline examination (Fig. 6). All participants were followed from the date of their baseline examination until December 31, 2007. The follow-up time was 22 years.

Postmenopausal osteoporotic women 1997-2003

Calcaneal QUS and DXA were performed in parallel annually for 7 years in eighty women, 53-73 years, with postmenopausal osteoporosis. The women were recruited from the Endocrine outpatient clinic or via advertisement in the local newspaper if they had known primary osteoporosis and/or fractures, and were treated with oestrogen hormone replacement (HRT), calcium 1000 mg and vitamin D 800 units/day during the last 6 months. Osteoporosis was defined according to the WHO as BMD lower than –2.5 SD of young adults (T-score) from the LUNAR USA reference population of the same gender, measured at the lumbar spine using DXA. Due to difficulties of recruiting 80 women who fulfilled these criteria, 3 patients with BMD = –2 SDs as T-score but with at least one osteoporotic fracture were included. The mean L2-L4 BMD T-score at start of the present study was – 2.6 SDs and 60% had suffered osteoporotic fractures. There were 39 radial, 22 vertebral, 7 rib, 8 ankle, 9 hip and 2 upper arm fractures in 48 patients. The oldest woman died just before the two-year examination; hence, 79 women were followed up for 7 years (Fig. 6).

The Gothenburg WHO MONICA study 1995 and re-examination 2008

A random population sample of 1200 men and 1200 women, aged 25-64 at baseline, was recruited from the third population screening in WHO MONICA, Gothenburg, Sweden. The participation rate varied from 52% (young men) to 82% (older women). In total, 1616 men (n=746) and women (n=870) participated. Fractures and CVD were captured until December 31, 2008, from the Swedish Hospital Register via the Swedish National Board of Health and Welfare, Stockholm, Sweden. The follow-up time was 13 years. In 1995, every 4th participant was selected for bone measurement and extensive hormone blood sampling. Additionally, QUS was carried out on all women in the age groups 45-54 and 55-64 years, in total 662 subjects. Bone measurements and blood samples were available from 410 subjects (i.e., a 73% participation rate; 58% men and 86% women); 96 men and 314 women, at the re-evaluation in 2008, Fig. 6 and Fig. 7 (flow chart).
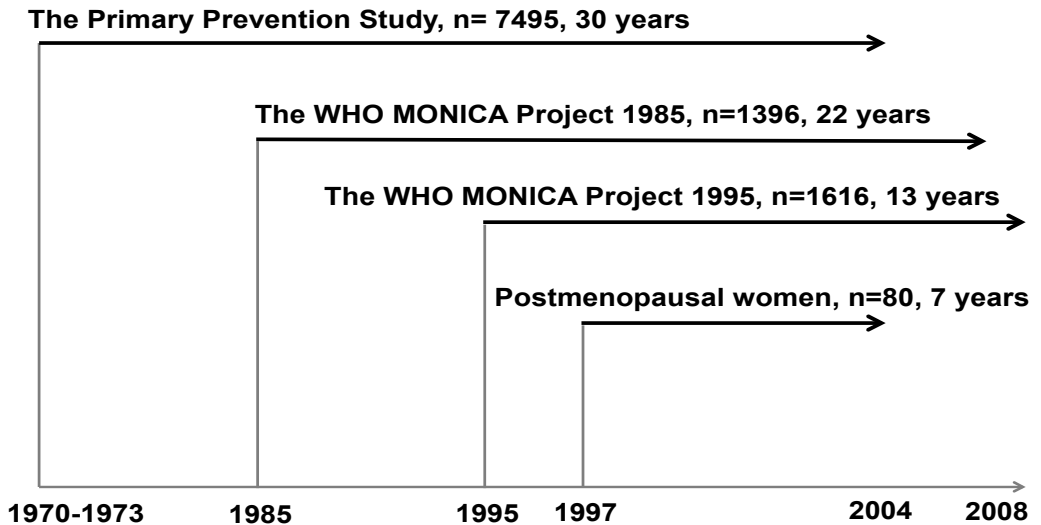
The Primary Prevention Study, n= 7495, 30 years

The WHO MONICA Project 1985, n=1396, 22 years

The WHO MONICA Project 1995, n=1616, 13 years

Postmenopausal women, n=80, 7 years

1970-1973   1985   1995 1997   2004   2008

Fig. 6 The diagram is a description of the follow-up duration with respect to the different study populations in all of the papers in this thesis.
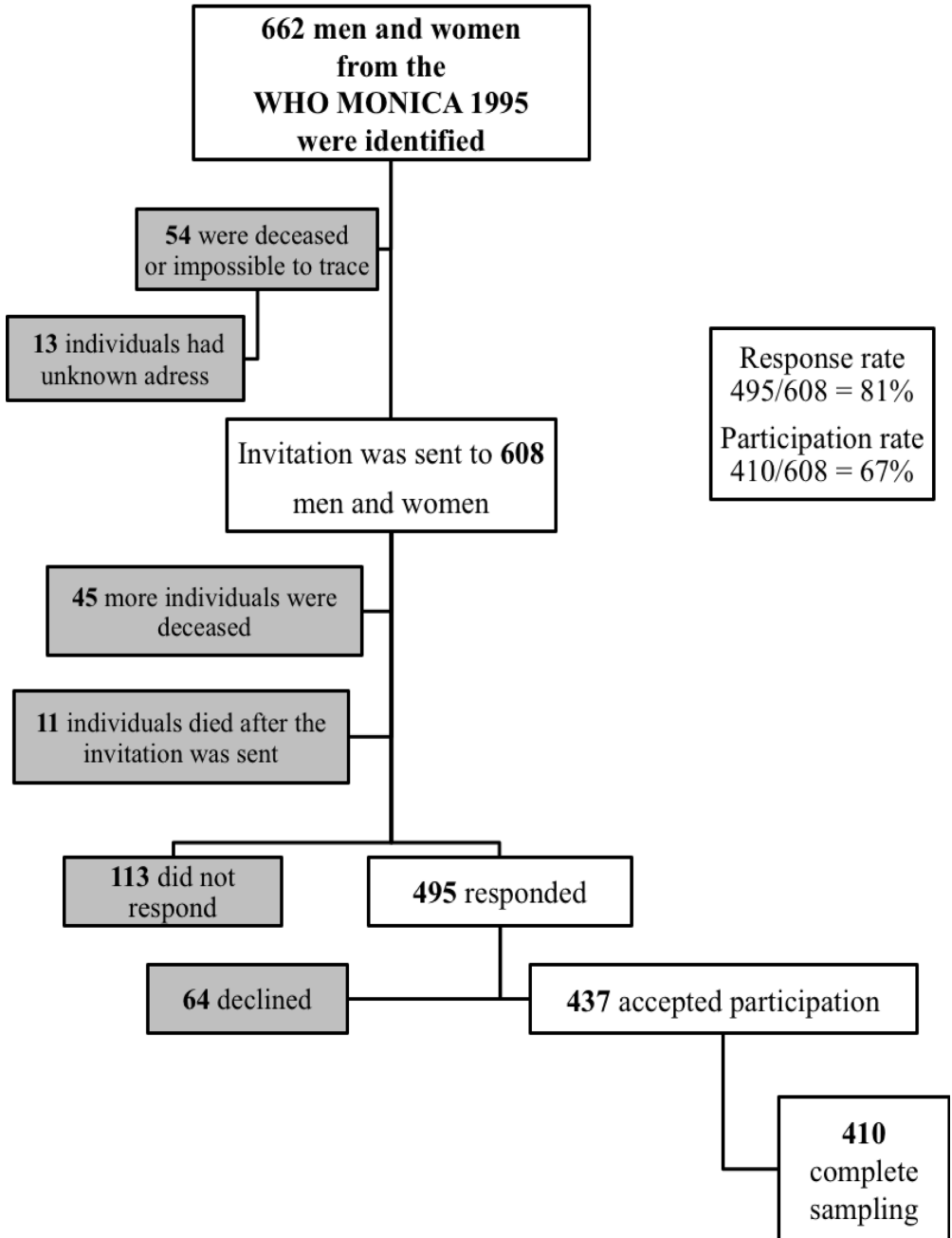
```
                    ┌─────────────────────┐
                    │   662 men and women │
                    │      from the       │
                    │  WHO MONICA 1995    │
                    │   were identified   │
                    └─────────────────────┘
```

662 men and women from the WHO MONICA 1995 were identified

54 were deceased or impossible to trace

13 individuals had unknown adress

Invitation was sent to **608** men and women

Response rate
495/608 = 81%

Participation rate
410/608 = 67%

45 more individuals were deceased

11 individuals died after the invitation was sent

113 did not respond

495 responded

64 declined

437 accepted participation

410 complete sampling

Fig. 7 Flow chart of the 13-year follow-up of 662 individuals with QUS and hormones from the WHO MONICA 1995 cohort and re-examined in 2008.

Table 3 General characteristics of the different populations in this thesis.

	The Primary Prevention Study	WHO MONICA 1985	Post-menopausal women	WHO MONICA 1995	WHO MONICA 2008
Population size	7495	1396	80	1616	410
Sex, (% women)	Men	Men and Women (53%)	Women	Men and Women (54%)	Men and Women (77%)
Age at entry, years	47-55	25-64	53-73	25-64	25-64
Participation rate, %	75	71	100	68	67
Fractured during follow-up, %	14	10	23	13	26
HRT users, %	0	33	100	31	8
Calcium/vitamin D, %	-	<1	100	0	10
Lipid lowering, %	<1	<1	0	0	15

Anthropometry

Body weight was measured to the nearest 0.1 kg in the fasting state with the subject in underwear and without shoes. Body height was measured without shoes to the nearest 1.0 cm. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m2). Waist circumference was measured with a soft tape midway between the lowest rib margin and the iliac crest in the standing position. The hip circumference was measured over the widest part of the gluteal region and the waist/hip circumference ratio (WHR) was calculated. A single operator performed the measurements at each examination.

Blood pressure

Paper I and II: Blood pressure was measured twice to the nearest 2 mm Hg on the right arm in the sitting position, after 10 minutes' rest. Disappearance of Korotkoff sounds (phase V) was used to determine diastolic blood pressure. A random zero blood pressure devise (Hawksley & Sons, Lancing, United Kingdom) was used. A cuff size corresponding to the circumference of the right arm was chosen.

Physical activity at work and during leisure time was coded from 1 to 4, with 1 denoting sedentary work or leisure time activity, and 4 denoting very heavy work or strenuous leisure time activity. This scoring system was developed by Saltin & Grimby (102).

Physical activity

Work   **Leisure**

Grade 1

Mainly sedentary work

Complete inactivity during leisure time, e.g., watching TV

Grade 2

Some walking and standing e.g., teachers, light tool and machinery workers

Moderate physical activity for at least 4 hours/week, e.g., cycling, walking to work, gardening

Grade 3

Generally walking with some lifting, e.g., postmen and heavy tool and machinery workers

Regular, more strenuous activity, e.g., running, tennis, heavy gardening

Grade 4

Heavy manual work, e.g., lumberjacks, dock and farm workers

Regular hard physical activity several times per week

Fig. 8 An illustration of the various grades of physical activity at work and during leisure time based on the questionnaire by Grimby and Saltin. Reprinted with the kind permission of Lars Wilhelmsen.

Smoking habits were initially coded as 1 = never smoked; 2 = former smokers of more than 1 month's duration; 3 = smoking 1-14 cigarettes per day; 4 = smoking 15-24 cigarettes per day; and 5 = smoking 25 or more cigarettes per day, in *Paper I*, and current smokers were merged into one group. In *Papers II, IV* and *V*, smoking habits were coded as 1 = current smokers, 2 = former smokers, and 3 = non-smokers.

The number of cups of coffee consumed per day was recorded. In *Paper I*, the different consumption levels were merged into only non-drinkers and coffee drinkers.

Psychological stress, defined as feeling tense, irritated, nervous, anxious, or having sleep disturbances due to problems at home or at work, was rated from 1 to 6, with 1 = no stress experience, 2 = experiencing some stress periods at some point, 3 = some stress periods during the last 5 years, 4 = several stress periods during the last 5 years, 5 = continuous stress during the last year, and 6 = continuous stress during the last 5 years. For the analysis, grades 1-2, 3-4, and 5-6 were combined.

Alcohol abuse was dichotomised by the presence or absence of registration with the Gothenburg Board of Social Welfare for medical or legal problems attributed to alcohol (103).

Occupational class was based on current occupation, as recorded in the questionnaire, and was further ascertained at the screening examination. The original data on occupation were reclassified according to a socio-economic classification system elaborated by the Swedish Central Bureau of Statistics, described in detail by Rosengren et al. (104).

In *Paper I*, the highest class (employed and self-employed professionals, higher civil servants, and executives) is denoted as 1 and the lowest class (unskilled and semi-skilled workers in goods and service production; for example, industrial workers, dockers, lorry drivers) is denoted as 5.

Fractures

Records of X-ray verified fractures deemed to be of mainly osteoporotic origin (upper arm, wrist, ankle, leg, hip, pelvis, rib, vertebrae and foot), according to the International Classification of Diseases (ICD) 9 codes 805-825 and E885-E888, and ICD 10 codes S07, S12, S22, S32, S42, S52, S62, S72, S82, S92, T08, T10, T12, and T14 during 30, 20 and 13 years (1970-2008) were retrieved from the Gothenburg hospital registers via the National Board of Health and Welfare, Stockholm, Sweden.

Questionnaires regarding the number and type of fractures and how they occurred during life were also assessed. Low energy fractures were regarded as possible osteoporotic fractures, whereas other fractures related to accidents were not included.

Pharmacological treatment

Information on ongoing pharmacological treatment was asked for in 1985, 1995 and 2008 with similar questionnaires and coded according to the Anatomical Therapeutic Chemical (ATC) Classification System. In paper IV, the N group is defined as tranquilisers but comprised tranquilisers, sedatives, antidepressants, and central nervous system-acting analgesics.

Bone measurements

Quantitative Ultrasound Measurement (QUS)

QUS (LUNAR Achilles, Madison, WI, USA) was performed using water-based devices on the right os calcaneus with the subject in the sitting position. The heel was placed in a bath of water with a temperature of 37°C between two ultrasonic transducers. The ultrasound uses high-frequency sound waves to measure heel bone, using the velocity of the ultrasound signal (Speed Of Sound=SOS) and the frequency attenuation (Broadband Ultrasound Attenuation=BUA). SOS and BUA are combined by the manufacturer to form an index called stiffness, which is

expressed as a percentage of the result from young adults (peak bone mass), according to the manufacturer. The procedure took 20 minutes per subject. The same operator performed the ultrasound measurements with the same QUS device throughout each study *(Paper III, IV and V)*. The QUS device was subjected to service and software updates by authorised personnel according to the manufacturers recommendations.

The standard error of a single determination was assessed according to the formula: $\sqrt{[(\Sigma di^2)/2n]}$, where Σdi^2 is the sum of individual differences squared, and n is the number of observations. The SOS varied between 1,441 and 1,584 m^{-1}; standard error (SE) 3.71 (0.25%), in 36 subjects aged 34-66 years who were examined twice with an interval of 1 h and the subjects walking around between examinations [105]. In these subjects, BUA varied between 80 and 138 db MHz^{-1}; standard error 2.20 (2.18%), and stiffness varied between 84% and 142%, standard error 1.85 (2.77%).

Fig. 9 The QUS (left) and the DXA (right) devices that were used in the various studies in this thesis. Printed with permission of the two operators.

Dual energy X-ray Absorptiometry (DXA)
Body mineral density (BMD) (g/cm^2) and body mineral content (BMC) (kg) were measured with DXA (LUNAR DPX-L, Lunar Radiation Inc., Madison, WI, USA), including total body, lumbar spine (anterior-posterior L_2-L_4), femoral neck and distal

radius. LUNAR software was used for scanning (version 1.33) and analysis (version 1.33). The in-house precision errors on the scanner used (system 7156), as determined from duplicate examinations in 10 healthy subjects, were 1.46% for total body BMD, 0.81% for anterior-posterior spine BMD, 1.25% for femoral neck BMD and 1.66% for forearm BMD. The corresponding variation for total body BMC was 1.94%. The reference database used was the LUNAR USA reference population for the region examined. A quality assurance test with a phantom was performed every day and with a European phantom once a year. The SD for repeated measures was 0.01 g/cm^2 (1%) for L_2-L_4 and 0.015 g/cm^2 (1.5%) for the femoral neck during both short-term and long-term recordings. The same person performed all DXA measurements during the entire study period in *Paper III*.

Bioimpedance

Body composition was evaluated by impedance measurements (SEAC Multiple frequency bioimpedance meter model SFB 2, UniQuest Ltd, Queensland, Australia) based on resistance and reactance in the total body, *Paper III, IV, V*. Bioimpedance is based on the concept that lipid-rich tissues are more resistant to an electrical current than tissue rich in water and electrolytes. With this method the intracellular and extracellular resistance is calculated and the fat-free mass, body fat and lean body mass are derived on the basis of the given age, height and weight (106, 107). Although the assessment of minor changes in fat-free mass and body fat is limited, the method is considered as fairly reliable when performed in patients with stable water and electrolyte balance (108).

Biochemical analyses

Venous blood samples from an antecubital vein were drawn between 8 and 10 am after an overnight fast in all relevant studies. After centrifugation, serum and plasma aliquots were frozen in 1ml glass ampoules and stored at -70°C until analysed,

which took place within one year for all variables. Samples in menstruating women were collected on cycle day 7-9.

Concentrations of serum total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides were determined enzymatically (Boehringer, Mannheim, Germany). Fibrinogen was analysed according to a polymerisation method described by von Clauss (109).

Serum total testosterone was determined by a non-extraction competitive radioimmunoassay (RIA) (ICN Biochemicals Inc. Diagnostics Division, Costa Mesa, CA, USA). The coefficient of variation (CV) was 16.3 % for total testosterone levels at 2.0 nmol/l and 10.0% for total testosterone levels at 26.8 nmol/l. Serum total oestradiol was determined by RIA (Clinical Assays™ Estradiol-2, DiaSorin, Saluggia, Italy). The CV was 10.0 % for oestradiol levels at 0.4 pmol/l and 16.0 % for oestradiol levels at 0.04 pmol/l. Sex hormone-binding globulin (SHBG) was determined by Immunoradiometric Assay (IRMA) (Orion Diagnostica Oy, Espoo, Finland). The CV was 4.2 % for SHBG levels at 19.7 nmol/l and 6.3 % for SHBG levels at 76.3 nmol/l. Serum free testosterone was calculated according to Vermeulen et al. (110). Serum IGF-1 was determined by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) in 1995. The CV was 8% for IGF-1 at levels of 67 ug/l and 6% at 332 ug/l. In 2008 Siemens Immulite 2500 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) was used, which was 28% lower than Nichols RIA, which was withdrawn after 1995. The equation was; y= 1995 value x 0.7245+ 12.6245, r=0.9859 (n=138). Only converted values for IGF-1 are given.

Osteocalcin was determined using a double antibody radioimmunoassay method (International CIS, Gif-sur-Yvette, France). Total CV was 11% at 13.9 µg/l, 8% at 10.7 µg/l and 8.8% at 23.3 µg/l.

Serum intact parathyroid hormone (PTH) was determined by immunoradiometric assay (Nichols Institute Diagnostics). The intra-assay CV was 10% for the interval 4-10 ng/l, and 6% for the interval 11-30 ng/l; total CV was 6%.

STATISTICAL METHODS

All the papers in this thesis are observational studies, based on different cohorts. A "cohort" in epidemiology is defined as "any designated group of individuals who are followed over a period of time" (111). Models regarding survival analysis were used to study risk factors for fractures. In general, in survival analysis the outcome event of interest is the time until an event occurs (112). Two basic concepts of survival analysis are survival function and hazard function.

The survival function is the probability of not suffering the event of interest from the start of the study to a specified time in the future (112). The hazard function describes the momentary risk of an event, given that the individual has survived until that point (112).

The momentary risk of osteoporotic fracture is expected to depend on current bone quality variables and previous values of such variables are not expected to contribute appreciably to the risk. This implies, together with some general assumptions, that the predictive power of a bone quality variable determined at a baseline examination normally decreases with time. One might say that a bone quality variable constitutes a predictor of osteoporotic fractures if it is able to predict future bone quality. A risk variable is thus more strongly related to future bone quality than to present bone quality if its predictive power increases with time. A risk variable that affects bone quality slowly could behave in such a manner.

The association between the hazard function and some risk factors was explored using either the Cox proportional hazard model (113) or the Poisson regression model (114). The Cox regression model requires that the hazard ratio (HR), which is the relative hazard between two groups, is independent of time (113). The Poisson regression is a model used to estimate a continuous hazard function (114). In addition, in *Paper II*, the smoothing spline method was used. The model fits a smooth curve on a set of observations in order to reduce the "noise," thereby increasing the transparency of the data and allowing for better statistical and clinical

interpretation (115). To characterise the predictive ability of a fracture risk factor, the gradient of risk per one SD was used. The gradient of risk per one SD of a predictor is the HR between two individuals, who differ by one SD with respect to the predictor, but are equal with respect to other variables (82).

Statistics in Paper I

The main objective of the study was to examine to what extent factors measured at baseline in 1970-1973 (at age 46-56 years) predicted the incidence of hip fracture over a 30-year follow-up period, until 2003, which extended into advanced age. Subjects with missing values for baseline factors were excluded from the analysis. The association between HR for fractures and risk factors were modelled using the Cox regression and Poisson regression analyses. The change over time in risk factors was analysed in a time-dependent analysis. The gradient of risk per one SD was used.

Statistics in Paper II

Mean values, SDs and confidence intervals (CI) were calculated using conventional methods. The Student's t-test and chi-square test were used for a brief comparison between groups. A special type of Poisson regression analysis was performed in order to estimate continuous hazard functions, from which it is possible to calculate the HR over time for osteoporotic fractures for each risk factor, taking age and sex into account (114, 115). Only the first fracture was included in the analyses for subjects who sustained more than one fracture. The gradient of risk per one SD was used.

Statistics in Paper III

The mean and SD were calculated using conventional methods. Simple correlations were calculated using Pearson's method. Partial correlations with adjustment for age were performed. Multiple linear regression analyses were also performed using SOS,

BUA and stiffness as dependent variables in order to test independent associations. Variables were entered in a stepwise forward manner. Differences between groups were tested with ANOVA during 7 years. Differences of repeated measurements were tested using Duncan's ANOVA multiple range test.

Statistics in Paper IV

Means and SDs are shown for continuous variables. Correlations with age were calculated using the Spearman rank correlation. The age-adjusted odds ratio (OR) with a 95% CI was calculated. Multiple, logistic regression models were used to test the interaction between factors. Variables were entered in a forward manner. In order to further elucidate the influence of stroke on the risk of fracture we estimated the hazard function of fracture from the stroke event (after baseline 1995) to the end of 2008 or to death, if that occurred earlier. The fracture hazard function before or without stroke was estimated for comparison. For both hazard functions the variables time since baseline and current age were included. Changes between fractured and non-fractured groups in 1995 and 2008 were tested with the Mann-Whitney U-test for continuous data, Fisher's Exact test for dichotomous variables, and Mantel- Haenszel's Chi Square test for ordered categorical variables.

In the subgroup, comparison of intraindividual changes between 1995 and 2008 was perfomed with the Wilcoxon sign rank test.

Statistics in Paper V

Means and SDs were calculated using conventional methods. For comparison between groups, the Mantel-Haenzel Chi Square test was used for ordered categorical variables and the Mann-Whitney U-test for continuous variables. Odds ratios (OR) were calculated using the chi-square test for the analysis of proportions between groups.

RESULTS

Results - Paper I

In total, 2186 fractures had occurred in 1031 out of 7495 men (14%) during 30 years; the fracture distribution is shown in Fig. 10.

In total, 451 of 7495 (6%) men sustained a hip fracture, Fig. 11. Men who had died from cancer within 2 years of their hip fracture (n=39) were excluded from the analysis in order to avoid including pathological fractures due to metastatic disease.
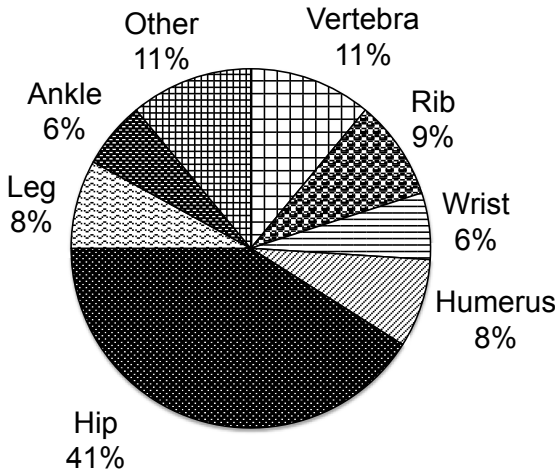
Fig. 10 Distribution of 2186 fractures in 1031 men during 30-year follow-up of 7495 men, start in 1970.
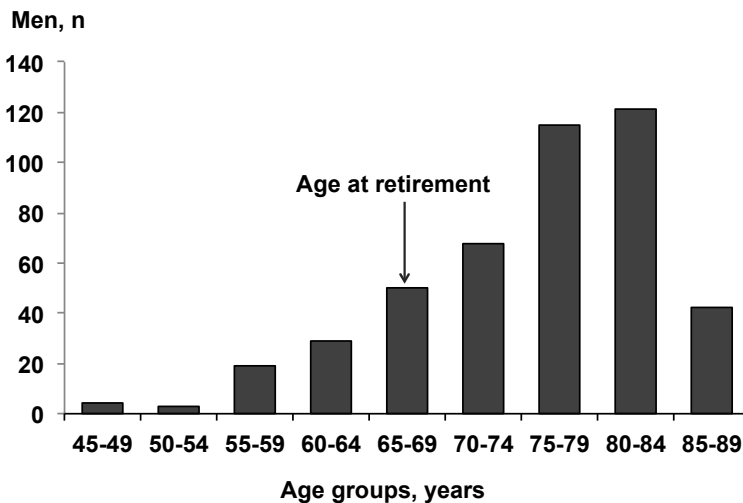
Fig. 11 Distribution of 451 hip fractures in various age groups of men during 30 years, start in 1970.

Variables independently associated with hip fractures are shown in Table 4. High degree of leisure-time physical activity, high occupational class, and high BMI protected against hip fracture. However, work-related physical activity was not protective. Smoking, tall stature and interim stroke or dementia increased the risk.

Table 4 Factors independently associated with the incidence of hip fractures during 30 years of follow-up of 7495 men according to multivariable Cox analysis.

Variable	Parameter estimate	Standard Error	P-value	Hazard Ratio	95 % Confidence Interval
Body mass index	-0.061	0.018	0.0008	0.941	0.908 - 0.975
Physical activity, leisure	-0.192	0.081	0.017	0.826	0.705 - 0.967
Age	0.080	0.024	0.0010	1.084	1.033 - 1.137
Height	0.036	0.008	<0.0001	1.037	1.020 - 1.053
Low occupational class	0.158	0.046	0.0005	1.171	1.071 - 1.281
Smoking	0.302	0.105	0.0040	1.352	1.101-1.661
Alcoholic intemperance	0.600	0.106	<0.0001	1.821	1.473 - 2.252
Stroke before fracture	0.762	0.143	<0.0001	2.143	1.618 - 2.839
Dementia before fracture	1.639	0.180	<0.0001	5.150	3.621 - 7.326

For the risk score of hip fracture determined in the present study, the gradient of risk as a function of time since measurement was calculated with corresponding 95% confidence intervals (Fig. 12). This risk function was based on variables in Table 4, but alcoholic intemperance, stroke and dementia before the fracture were excluded. A high gradient of risk was seen soon after the baseline measurement. The gradient decreased significantly ($p<0.001$) during the latter part of the time range, but the hazard associated with the risk function was still 2.0, 20 years after baseline.
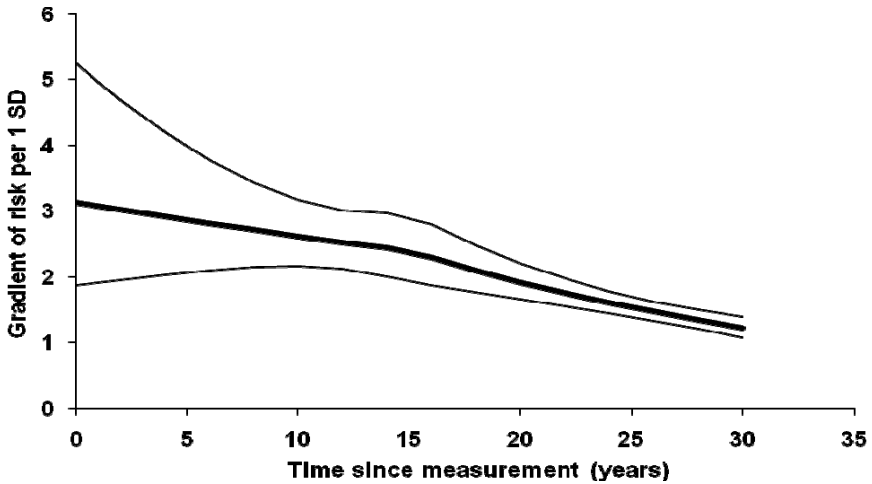

Fig. 12 Gradient of risk per one SD of the significant risk factors for hip fractures in *Paper I.* The bold curves show the estimated gradient of risk per 1 SD and the thin lines the 95% confidence intervals.

Results - Paper II

A total of 258 osteoporotic fractures occurred in 143 individuals out of 1396 (10%). The distribution of these fractures is shown in Fig. 13.
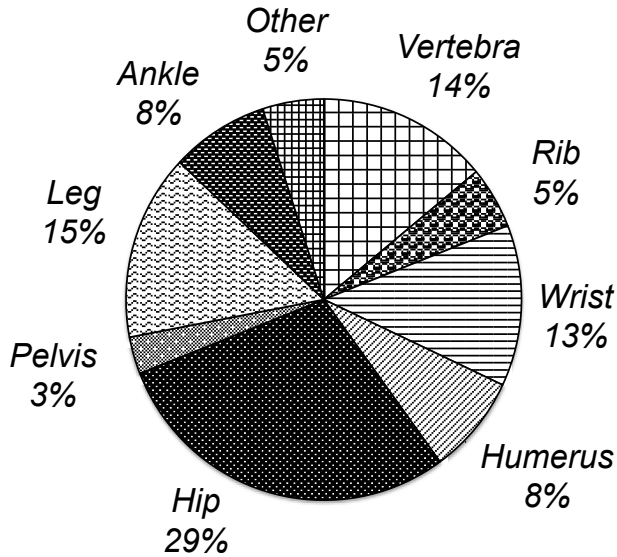

Fig. 13
Distribution of 258 fractures in 143 individuals during 20-year follow-up of 1396 men and women, start in 1985.

Previous fracture, smoking, coffee consumption, cholesterol, and lower BMI increased the risk of fracture independently of age and sex. Using a spline Poisson regression analysis adjusted for age, sex, and BMI, a significant increase in the gradient of risk for cholesterol by time (p=0.0271) was shown, Fig 14.
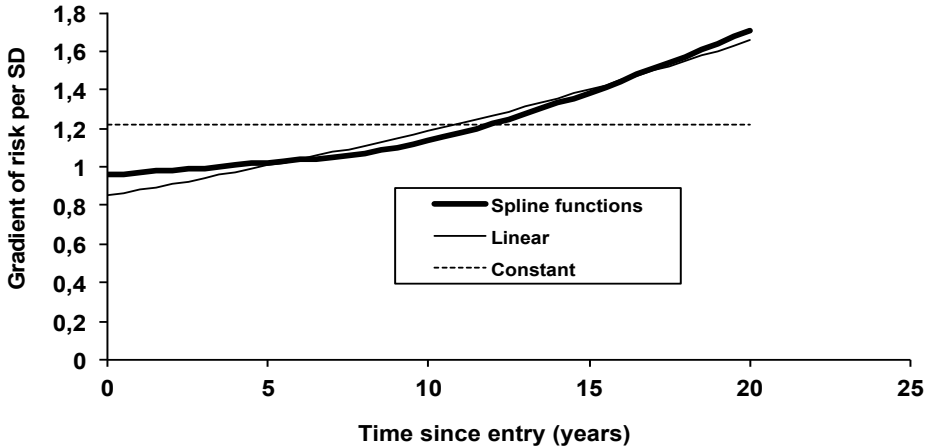
Fig. 14 Serum total cholesterol as an osteoporotic fracture predictor as a function of time. The dotted line shows the risk gradient as being constant over time (Cox's proportional hazard model). The thin solid line shows the gradient of risk per one SD of serum total cholesterol when the logarithm is linear as a function of time (Poisson regression model). The thick solid line shows the gradient of risk according to smoothing spline functions (Poisson regression model).

Results - Paper III

Osteoporosis, determined as a T-score <−2.5 SD, was found at baseline in 70% and 56% with QUS and DXA, respectively, in 80 osteoporotic women with osteoporosis and/or fractures. The corresponding figures at the seven-year follow-up were 74% and 65%, respectively. Osteoporosis and osteopaenia were found in 94% measured with QUS and 98% measured with DXA at baseline and in 93% and 95%, respectively, at 7 years.

Sensitivity was calculated as the number of subjects with t-score <-2.5 SD according to QUS/ the number of subjects with t-score <-2.5 SD according to DXA.

Specificity was calculated as the number of subjects with T-score >-2.5 SD according to QUS/the number of subjects with T-score >-2.5 SD according to DXA. The sensitivity for QUS compared with DXA as the "gold standard" ranged from 76% to 84% at repeated measurements during 0–7 years, and the specificity ranged from 36% to 57% during the same time period.

A QUS T-score <−3.65, corresponding to a stiffness index <60% of young adults, was consistent with a T-score of <−2.5 SD at DXA. This corresponded to SOS levels <1485m/s and BUA <96 db/MHz measured with QUS.
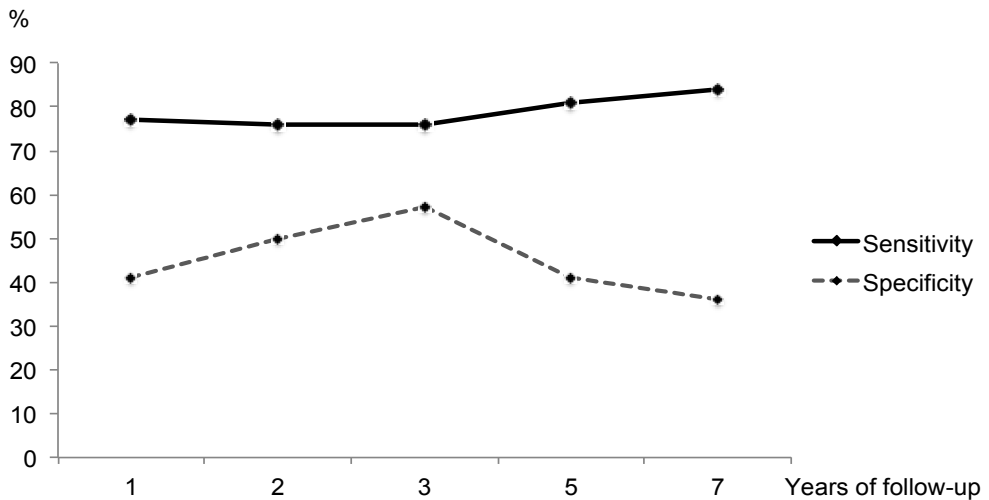

Fig. 15 Sensitivity and specificity of QUS against DXA in diagnosing osteoporosis according to the operational definition of osteoporosis.

Results - Paper IV

Since 1995, 321 fractures had occurred in 210 out of 1616 subjects (13%), (women 15%, men 10%) during 13 years. The fracture distribution is shown in Fig. 16.
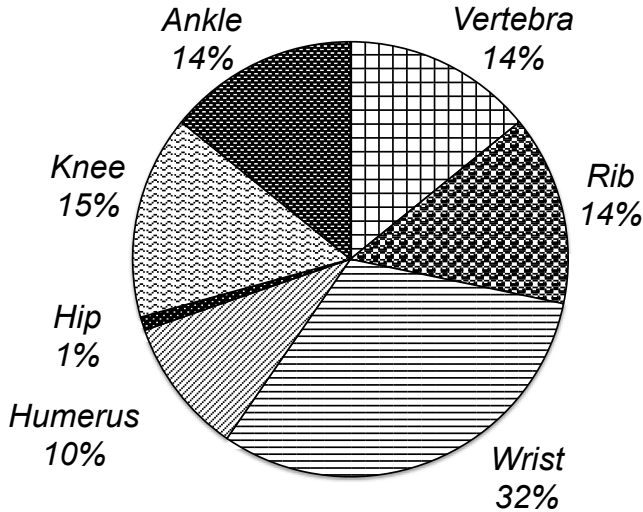
Fig. 16 Distribution of 321 fractures in 210 subjects, during 13-year follow-up of 1616 men and women, start in 1995.

Stroke before 1995, physical inactivity during leisure time, age, female sex and fewer previous fracture were independent predictors of future fracture.

Among subjects with fractures, more CVD, mainly stroke, higher cholesterol levels but less lipid-lowering treatment, higher blood pressure and fibrinogen levels, lower QUS and lower physical activity during leisure time were found. More use of tranquilisers and, in women, low oestradiol levels were present in fractured compared with non-fractured subjects.

The age-adjusted ORs showed that subjects with fractures were more often women, had lower height, QUS, physical activity during leisure time and IGF-1, higher cholesterol, triglycerides and more myocardial infarctions and stroke than those who did not fracture. In women, serum oestradiol was lower in those with fractures. Serum oestradiol or testosterone in men did not differ between those who fractured

and those who did not. In a forward stepwise linear regression analysis, only higher age, lower QUS and lower physical activity during leisure time were independently associated with fractures.

HRT had decreased from 31% to 8% during follow-up and lipid-lowering and anti-osteoporotic agents had been introduced. However, the latter were used less than expected in fractured subjects.

Results - Paper V

Secular trends in sex hormones were seen in men of similar age, 13 years apart, with lower serum free testosterone in all men in 2008 compared with men of similar age in 1995. Body weight, physical activity and fracture incidence were unaltered.

All women in 2008 had higher fracture incidence, 26% vs 14% (p=0.00003), than women of similar age in 1995. The vertebral compressions in postmenopausal women of 2008 was higher, 19% vs 8% (p=0.031), than in women of 1995.

Women aged 45-54 years had lower serum oestradiol in 2008 mainly due to withdrawal of HRT (decreased from 35% to 8%). These women had higher physical activity during leisure time and higher bone mass and lower physical activity at work. Serum lipids had declined in all men and women with time, irrespective of the use of lipid-lowering agents.

DISCUSSION

Physical inactivity, smoking, high cholesterol and stroke were independent risk factors for fracture, indicative of a link between metabolic bone disease and CVD.

The mentioned risk factors are modifiable but non-modifiable risk factors, such as high age and female sex, were also found. Secular trends were found in sex hormones and fractures in men and women.

Physical activity and fractures

The main finding in *Papers I and IV* was undoubtedly the effect of physical activity, a modifiable protective factor against future fractures.

In *Paper I*, a follow-up period as long as 30 years for men revealed that physical activity during leisure time was the strongest protective factor against hip fractures, along with high occupational class and high BMI. This effect was true not only in the univariate analysis of potential risk factors, but persisted in the multivariate analysis independently of other factors. The effect of physical activity on bone density has been shown to be beneficial in all ages (116, 117). Exercise during late adulthood was associated with less bone loss in postmenopausal women (118). Exercise early in life was associated with higher bone density in men (119), although the activity has to be continuous throughout life (120). Hence, it never seems to be too late in life to change to a more active leisure time. Leisure time physical activity is mainly performed outdoors whilst it correlated positively with vitamin D, which is beneficial for bone synthesis (58).

In order to maintain a good skeletal condition and reduce the risk of falling, balance training, improved muscle strength and reflexes are important to reducing the fall risk (121, 122). A longitudinal study in men of similar age by Michaelsson et al. (123) also showed a protective effect of exercise against hip fractures. Similar and supportive results are also found in other studies (124-128).

However, physical activity during work had the opposite effect in *Paper I*. According

to the questionnaire used, the higher the degree of physical activity at work, the heavier were the weight-lifting activities. On the other hand, men with high physical activity at work also had lower occupational class (coded as 1 for the highest, and 5 for the lowest) and a greater frequency of alcohol intemperance. When adjusting for those factors it was revealed that physical activity at work did not have a clear association with hip fracture, but was rather mediated by lower occupational class and alcohol abuse.

Men who were physically active during their leisure time were of a higher occupational class, and were less often registered for alcohol abuse. Thus, the decreased fracture incidence in physically active men could be explained, in part, by these factors. Yet, in the multivariable analysis, high leisure time physical activity was still associated with a lower risk of hip fracture. Earlier analyses have also found that leisure time physical activity protects against fractures (123, 125-130).

To our knowledge, no other studies have reported a difference with regard to the fracture risk between leisure time activity and work activity over such a long follow-up period. Recently, Moayyeri et al. (131) showed that leisure time physical activity decreased the HR of hip fracture in men but physical activity at work did not affect the risk. That study comprised 6514 men with a mean age of 62 years who were followed for 7.5 years; thus, a younger cohort and shorter follow-up time than in our cohort.

In *Paper IV*, a 13-year follow-up study, confirmed the results of the 30-year follow-up concerning physical activity. In the whole cohort of 1616 men and women, higher leisure time physical activity decreased the risk of future osteoporotic fracture, which persisted in the multivariable logistic regression analysis, as well.

In this young cohort, 25-64 at study start, the fracture panorama was different compared with *Paper I,* with only a few hip fractures. The majority of fractures were radial, knee, and ankle, and could be explained by the well-preserved reflexes in younger individuals. Physical activity and fractures had increased in 2008 and a more active lifestyle may have contributed to more accidents and falls (132). This is also in

accordance with other studies reporting the same effect on osteoporotic fractures (130, 131).

Physical inactivity has been considered as a risk factor for CVD outcomes (63, 65), especially in women (64). It is now shown that it is a risk factor for fragility fractures as well, the effect of which is present at different ages in both men and women.

High cholesterol and fractures

In *Paper II*, during 20 years of follow-up, serum total cholesterol was identified as an independent osteoporotic fracture risk factor, with a predictive power that improves with time. The importance of cholesterol is so far underestimated. This could open up a new dimension of the fracture risk assessment.

These findings are in line with and support the results in *Paper I*, in the sense that hard CVD end points, such as stroke and dementia, are strong predictive risk factors for hip fractures among elderly men. Thus, serum total cholesterol is a risk factor not only for vascular-associated diseases, but also for osteoporotic fractures, perhaps pointing towards a common long-term origin of metabolic bone and cardiovascular disease.

As cholesterol's predictive power improves with time and does not have the same effect at any given time, it is reasonable to conclude that only studies with long follow-up times will be able to assess the true power of cholesterol as an osteoporotic fracture risk factor. In this perspective, it is very difficult to evaluate studies of shorter duration aimed at investigating cholesterol as an independent osteoporotic fracture risk factor (133, 134), as well as lipid-lowering studies, which have been based on follow-up times of maximally 5 years (135, 136).

Some researchers have demonstrated an indirect association between serum total cholesterol and BMD, which is a major determinant of fragility fractures (137-141). To explain this association, some authors came to the conclusion that it was the result

of oestrogen deficiency rather than the cholesterol *per se*, although prospective studies are still warranted. This 20-year longitudinal study supports the notion that serum total cholesterol plays a role in causing osteoporotic fractures independently of gender and HRT. This is in line with a study by Wilhelmsen et al. (142), who reported that serum total cholesterol increases with age independently of gender, and Reinmark et al. (143), who reported an association between statin prescriptions and risk of hip fracture independently of gender in a population-based case control study.

It will be of major interest to study future fracture incidence as serum cholesterol decreases in the general population even without lipid-lowering treatment (144), and to determine whether lower cholesterol *per se*, independently of lipid-lowering agents, could result in a lower fracture incidence in the population. However, large studies, like 4S, LIPID, HPS, etc., reviewed by Rizzo et al. (145), have hitherto failed to show that statins prevent fractures. It has been debated whether the possibly beneficial effect of lower lipid levels on bone could be attributed to statins *per se* or to decreased cholesterol *per se*. A recent case control study showed a synergistic effect of statins and HRT on fractures but not from statins alone (146).

Stroke and fractures

Stroke and dementia, end points of CVD, predicted hip fractures in 7495 men at 30 years of follow-up in *Paper I*, and stroke predicted fractures in 1616 men and women in a 13-year follow-up period in *Paper IV*. A recent review collected evidence of an association between CVD and bone loss (147). However, the majority of the studies referred to were cross-sectional. Among the prospective studies, only few had longer follow-up periods than 10 years and fracture as an outcome was rare (147). An association between low BMD and stroke in elderly women was seen during two years of follow-up (148), but very few studies have found an association between fractures and stroke.

The results from *Papers I, II and IV* may indicate a link between metabolic bone disease and CVD. Stroke has been found to increase the risk of hip fracture 1.5 to 4-fold (149, 150). The risk of an osteoporotic fracture was found to be increased after both a diagnosis of coronary heart disease (CHD) (HR 2.32) and of stroke (HR 5.09) in 20-year follow-up of 30 000 twins (151).

In a population-based case control study of both men and women Pouwels et al. found a two-fold increased risk of hip fracture in patients with stroke at any time before fracture, but the risk was highest in patients who suffered a stroke within 3 months before the hip fracture (152). It has previously been shown that the risk of an osteoporotic fracture is higher during the first year after a stroke (153), which is in line with *Paper IV*.

The finding of more CVD, mainly stroke, and higher blood pressure, cholesterol, triglycerides and fibrinogen levels in fractured subjects may indicate a vascular pathogenesis in the development of fractures. It is tempting to speculate that a microangiopathy process is present also in bone. As in atherosclerosis, increased amounts of lipids accumulate in perivascular areas in bone (154), and a series of pathophysiological mechanisms seem to have a common role in the development of atherosclerosis and osteoporosis (154). This hypothesis might also explain why smoking is a strong risk factor for fractures (155-157). As seen in *Paper IV*, smoking had decreased to 11% in 2008 and was no longer a risk factor for fracture. Furthermore, the above hypothesis is strengthened by the inverse relationship between changes in QUS and cholesterol in *Paper IV*. A recent study on the estimated probability in women with CVD showed an increased risk of osteoporotic fractures (158).

Socioeconomic status

In *Paper I*, men of low occupational status had an increased fracture risk. This was not

explained by their slightly higher smoking rates (104, 159), by alcohol abuse, or by intercurrent stroke or dementia. Few studies have investigated socioeconomic status as a potential risk factor for fractures. A British report found that people of lower socioeconomic status had a higher risk of falling but no increased risk of hip fractures (160). Marmot (161) has shown that socioeconomic factors, denoted the status syndrome, are independent risk factors for various diseases, and the present results indicate that socioeconomic factors are also risk factors for osteoporotic fractures. A recent study by Mattila et al. (162) on socioeconomic status and fractures in young adult men (median age 19 years) showed that living in an urban area (town) increased the risk of fractures but that the level of education was not associated with an increased risk.

On the other hand, the incidence of osteoporotic fractures is higher in countries with economic prosperity, possibly due to decreased physical activity (57).

Pharmacological treatment and fractures

Fewer subjects with fractures had anti-osteoporotic agents than expected and fewer had lipid-lowering agents, in spite of higher serum cholesterol. This indicates under-treatment and also that osteoporotic patients are not properly taken care of in general. HRT use was present in 31% of the patients in the subgroup in 1995 and this figure had fallen to 8% in 2008.

The WHO MONICA 1995 cohort was probably one of the last treatment-naïve populations with regard to anti-osteoporotic treatment and lipid-lowering agents at study start in 1995. The pharmacological treatment both in 1995 and 2008 mirrors the underlying secular trends based on published results from large clinical trials regarding fracture prevention (163), CVD prevention (164), and risk of cancer due to HRT (165). HRT use in 1995 was very frequent and was used both for menopausal symptoms and as treatment for osteoporosis. HRT use has declined in the general population by 23%, leading to a decrease in serum oestradiol levels.

Use of tranquilisers and anti-hypertensives had increased in all subjects in 2008. These two major treatment categories are associated with a risk of vertigo, dizziness and falling.

Current smoking and fractures

Current smoking is a known risk factor for fracture and is partly BMD-dependent (155). Smoking was identified as a risk factor for fractures in men during a 30-year follow-up (*Paper I*) but also in both genders during a 20-year follow-up (*Paper II*). This is in line with our previous knowledge on risk factors for fractures (166, 167). Smoking declined in society from 26% to 11% between 1995 and 2008 and its effect on the future fracture incidence is unclear.

Age, female sex and fractures

Age contributes to the fracture risk independently of BMD. For a given BMD, the risk of fracture is higher in the elderly, compared with younger individuals (168, 169). Johnell et al. showed that the accuracy of BMD at predicting fractures at different sites is improved when age is taken into account. For instance, for hip fracture prediction the gradient of risk per SD decrease in femoral neck BMD was much higher in younger than in older men and women (170).

After the menopause, women are at increased risk of sustaining osteoporotic fractures, mainly due to the decrease in oestrogens (171). Female sex was clearly a risk factor for fracture, particularly pronounced in the 13-year follow-up, *Paper IV*. Age and female sex, however, are non-modifiable risk factors.

BMI and fractures

In *Papers I and II*, high BMI was protective against fracture independently of other factors. The risk ratio (RR), adjusted for age, for hip fracture is 1.95 when individuals with a BMI of 25 kg/m^2 were compared with those with a BMI of 20 kg/m^2 without taking BMD into account (172). However, the association with a BMI less than 20 kg/m^2 is dependent upon the BMD. Nevertheless, a modest risk persists after adjustment for BMD with respect to hip fractures (173). A comparison between 25 and 30 kg/m^2 is not associated with an expected decrease in risk, which is interpreted as leanness being a risk factor rather than high BMI being a protective factor (173).

Prior fracture as a risk factor for future fractures

The fracture risk is approximately doubled in the presence of a prior fracture (30). When BMD is taken into account, the RR is slightly decreased by approximately 10-15% (174). Prior fracture has been shown to be a risk factor for future fractures in *Paper II*, but this was not confirmed in *Paper IV*. However, in *Paper IV* fractures at ages below 40 years were, most probably, related to sports and not considered to be osteoporotic. On the other hand, a more active lifestyle in the elderly might have lead to more fractures (132).

Alcohol misuse and fractures

Alcohol abuse was found to be an independent risk of hip fractures in men, *Paper I*. The association between alcohol intake and the risk of fracture was dose-dependent and of a U-shape regarding the hip fracture risk in older men (175). Moderate alcohol consumption has not been shown to increase the fracture risk (176), whereas alcohol intake of three or more units per day results in an increased fracture risk (176, 177). The alcohol use in *Paper I* was heavy, as the data were collected from registrations by

the Gothenburg Board of Social Welfare for medical or legal problems.

Secular trends in hormones and fractures

Secular trends in sex hormones, with lower testosterone in men, were found. This is in line with a prospective study that showed an age-independent decline in serum testosterone in American men aged 45-79 years during 17 years (178). A Danish study of WHO MONICA cohorts also showed similar secular trends in corresponding ages to those in the present study (179). However, no secular trends in fracture incidence were found in men. Anthropometry could not explain the present results. The observed secular trend with decreased testosterone in men today is highly likely to be mirrored in the coming 10-20 years with an increased fracture incidence if the male sex hormone is of importance for bone structure. Secular trends in hip fractures are being discussed (180). Hip fractures are mainly due to falls and nutritional factors are important for the cortical bone.

Serum testosterone was higher in young women in 2008 compared with young women in 1995, and serum oestradiol was lower in the postmenopausal ones in 2008. The latter could be attributed to the almost total cessation of HRT in the past ten years. Secular trends in fracture incidence were seen in parallel in women, along with a higher incidence of vertebral fractures nowadays in *Paper V*. According to these results, the effect of HRT withdrawal seems to resulting rapidly in an increase in trabecular bone fractures, which are directly dependent on sex hormones. The results support the importance of oestrogen in the bone synthesis (171).

Nevertheless, serum lipids have declined in the general population during the last 15 years, irrespective of the use of lipid-lowering agents (144, 181). It will be of interest to see whether the lower blood lipid levels will result in a reduced fracture incidence in the long term, which would be in line with the results in *Paper II*.

Limitations

Limitations in Paper I

Registry data was used to identify end points. No hormones or bone markers were analysed. However, biochemical analyses available in the field of osteoporosis were limited in 1970.

Limitations in Paper II

The study lacks sufficient power to prove causal relationships; i.e., whether cholesterol causes myocardial infarction, which in turn, may increase the risk of falling. Nor was it possible to investigate other more specific mechanisms, such as local damage to the bone.

Limitations in Paper III

This was a study with a small sample size. However, good subject compliance (100%) was possible with repeated QUS and DXA measurements during 7 years. The number of sustained fractures at follow-up was small and fracture prediction with QUS based on the correlations with BMD or BMC was not possible.

Limitations in Paper IV

The population sample size was small and the follow-up of 13 years was relatively short. The age span is young, with few fracture end points, especially hip fractures.

Limitations in Paper V

The sample size for the hormone analyses was small, especially in 2008. Bioimpedance is not a fully reliable method, but the relationship between fat-free mass and fat mass could at least be studied. The RIA method for analysis of sex hormones is not trustworthy either, compared with mass spectometry, but it was the method of choice in 1995 worldwide. In order to test our hypothesis of possible secular trends it was necessary to use the same method also in 2008.

Strengths

Strengths in Paper I

The strengths of the study were the large cohort of men and the long follow-up period of more than 30 years. This is one of the longest reported follow-ups of fractures in men. Furthermore, the hip fractures were verified by X-ray.

Strengths in Paper II

The cohort was a random population sample of young, treatment-naïve men and women, who were subjected to a long follow-up time of 20 years with fracture end points.

Strengths in Paper III

The strengths of the study were its long duration, frequent measurements and no drop-outs, except for one death. All the women received similar osteoporotic treatment at start and osteomalacia and other possible secondary reasons for low BMD were excluded. The measurements were performed by the same person, and the same QUS and DXA devices were used throughout the study.

Strengths in Paper IV

All CVD incidents were verified through hospital registers and were strictly validated according to the WHO MONICA protocol and the fractures were verified by X-ray. We have captured the ages where many patients begin to develop fractures of osteoporotic origin and suffer from CVD events. In addition, the cohort was treatment-naïve regarding anti-osteoporotic and lipid-lowering agents at start in 1995.

Strenghts in Paper V

The setting and the devices were the same, except for the known change in IGF-1 method, in both examinations. Bone measurements with QUS were well correlated with DXA. However, for fractures we used the full cohort and matched comparable sample sizes of around 200 subjects in each age group.

CLINICAL IMPLICATIONS

How to prevent the first fracture – Primary prevention

It is of crucial importance that the general population adopts a healthier lifestyle and increases their general daily physical activity and outdoor activities. Of great importance - and a new finding for primary prevention - is that the recommended diet is similar to the one in CVD. Hence, lower cholesterol levels should be aimed at. Furthermore, in the same way as in CVD, smoking cessation should be strongly recommended. Low BMI should be avoided when reaching the "fracture" ages. Finally, it is up to the physician to recognise individuals at high risk of sustaining fractures in the same way as with CVD. A lot speaks in favour of common risk profiles with the exception of high BMI. Physicians who take care of stroke patients should be informed about the increased risk of fractures after an incident stroke. Special fall-preventive strategies should be advertised and promoted, such as trendy walking sticks and walking aids, so that younger stroke patients do not avoid taking advantage of such aids.
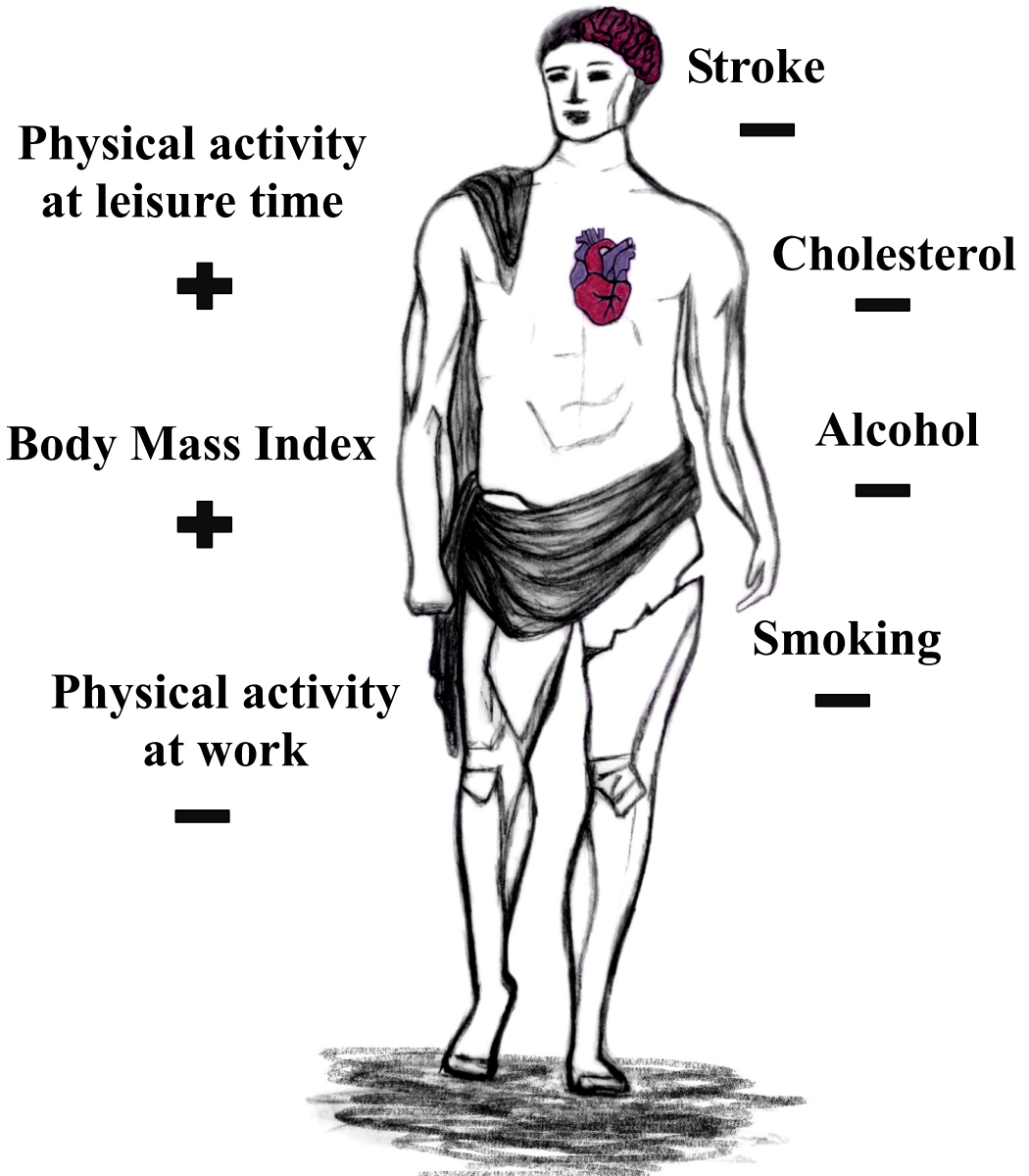
Nevertheless, a strong skeleton is built up during growth and a lifestyle with proper nutrition, physical activity and no smoking should be advertised in school as well to the younger generation. Even if there may be a common genetic denominator between osteoporosis and CVD (182), it is the chosen lifestyle that counts.

Physical activity, non-smoking and low cholesterol were factors of importance for reaching 90 years (183). Men and women could survive their the 100th birthday in the upright position with a maintained skeleton.

How to prevent the second fracture – Secondary prevention

Once a fracture has occurred, treatment should be initiated. A fracture chain should be developed and incorporated in the health system in each country so that all fractured individuals have the same chances to receive proper treatment and follow-up.

Modifiable risk factors for fractures
- a link between metabolic bone disease and CVD

**Physical activity
at leisure time**

+

Body Mass Index

+

**Physical activity
at work**

−

Stroke

−

Cholesterol

−

Alcohol

−

Smoking

−

CONCLUSION

Physical inactivity, smoking, high cholesterol and stroke were independent risk factors of fracture, indicative of a link between metabolic bone disease and CVD.

The mentioned risk factors are modifiable but non-modifiable risk factors, such as high age and female sex, were also found. Secular trends were found in sex hormones in both genders, and in women also in fracture type and incidence.

Lifestyle modifications that promote physical activity throughout life, non-smoking and low cholesterol, but avoid leanness and falling, could potentially prevent a significant proportion of fractures. Nutritional factors need to be better identified.

Primary prevention for cerebro-cardiovascular disease is also warranted for fracture prevention in the population.

Awareness among physicians is crucial in order to start treatment after a fracture as secondary prevention.

Take home message

Identify possible risk factors early in life!

You can modify your fracture risk!

It is never too late to change your lifestyle!

Avoid falling!

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