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OSTEOPOROSIS IN ELDERLY WOMEN IN PRIMARY HEALTH CARE

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ABSTRACT

Objective: All the studies are parts of the PRIMOS (Primary Health Care and Osteoporosis) project. *Study 1* investigates the relationship between central Dual X-ray Absorptiometry (DXA) measurements of the hip and spine and peripheral measurements of the calcaneus using Dual X-ray and Laser (DXL) technique. *Study II* investigates the association between the nutritional status of elderly free-living women, as determined by the Mini Nutritional Assessment (MNA) method, and the women's bone mineral density measured with DXA. *Study III* investigates the relationship to osteoporosis of calcium-regulating hormones and the IGF-I and IGFBP-1 status in the cohort. *Study IV* is an RCT evaluating the effect of ultra-low dose of estradiol on bone mineral density.

Methods: *Study population:* The participants in all studies come from the same population of approximately 940 women born between 1920 and 1930 living in the same primary care region in the southern part of Stockholm. Study I has 393 participants (388 included in the statistical analysis). Of these women, 351 were recruited by first inviting a random sample of 300, and then inviting all the rest of the women born 1926 and 1930. These 351 women are the population in study II and (with the exception of one woman excluded) in study III. The remaining 42 participants in study I and all 115 participants of study IV were included if eligible for a randomised controlled clinical trial (RCT) with estradiol. *The design of the RCT* was an open-label, randomised, parallel-group study with two treatment arms, one arm treated with a vaginal ring releasing 17 β-estradiol (average dose 7.5 µg/day) and a daily tablet containing 500 mg of calcium and 400 IU of vitamin D3, the other arm receiving treatment with 500 mg of calcium and 400 IU of vitamin D3. *Bone mineral density measurements*: The bone mineral densities (BMD) of the hip and lumbar spine (L1–L4) were determined using Hologic QDR 4500 equipment for DXA. The peripheral measurements on the calcaneus were performed with Calscan DEXA-T. *Mini Nutritional Assessment (MNA):* The nutritional status was determined with the MNA test consisting of 18 questions in four categories: anthropometric measurements, clinical and functional evaluations, assessment of dietary intake and self-assessment of health. The maximum score obtainable is 30 points, a score of <17 indicates malnutrition, 17–23.5 a risk of malnutrition and $\underline{\geq}$ 24 adequate nutritional status. *Laboratory measurements:* Parathyroid hormone (PTH), 25-hydroxy vitamin D, IGF-I, IGFBP-1, glucose and calcium-status were measured in all participants. Estradiol, SHBG, CTx, U-Dpd and other markers were followed in the RCT.

Results: Study I showed that measurements of the heel bone with DXL technique correlated fairly well to central measurements of the hip and spine on the group level. The same WHO cut-off point, –2.5 SD, was also applicable for the heel BMD when comparing with most central sites or combinations of sites with the exception of total hip. The change of reference population had a great influence on the amount of subjects classified as osteoporotic, which varied between 7% and 53% depending on the chosen reference population and site. Study II showed that women with an MNA score under the median score of 27 points had a twofold increased risk of having osteoporosis compared to women with MNA scores above the median. Very few women (7.4%) were assessed as at risk of malnutrition and only one woman was classified as malnourished. Study III showed a significant inverse relation of IGFBP-1 to the BMD values and a significant positive relation of IGF-I values to the BMD values at all sites with the exception of the lumbar spine. The use of loop diuretics was a more important cause of secondary hyperparathyroidism than the vitamin D status of the women. Study IV showed a small but significant effect on BMD of 7.5 µg/day estradiol administered through a vaginal ring during a follow-up of two years.

Conclusions: Bone mineral density measurements of the calcaneus with DXL technique correlate fairly well with central measurements. Adequate reference populations are important for *T*-scores. Elderly women with only a slight deterioration in their nutritional status have an increased risk of osteoporosis. IGF-I and IGFBP-1 are related to the BMD values. Secondary hyperparathyroidism may have other more clinically important causes than the vitamin D status in elderly women, i.e. treatment with loop diuretics. Estradiol doses of 7.5 µg/day seem to have a small but significant effect on BMD of elderly women.

Keywords: bone mineral density, DXL technique, elderly women, Mini Nutritional Assessment, nutritional status, osteoporosis, low-potency estrogen, growth factors, secondary hyperparathyroidism

LIST OF PUBLICATIONS

I Salminen H, Sääf M, Ringertz H, Strender L-E.
Bone mineral density measurement in the calcaneus with DXL: comparison with hip and spine measurements in a cross-sectional study of an elderly female population. *Osteoporosis International* 2005; 16(5):541–51.

II Salminen H, Sääf M, Johansson S-E, Ringertz H, Strender L-E.
Nutritional status, as determined by the Mini Nutritional Assessment, and osteoporosis: a cross-sectional study of an elderly female population. *Eur J Clin Nutr* 2006; 60(4); 486–93.

III Salminen H, Sääf M, Ringertz H, Strender L-E.
The role of IGF-I and IGFBP-1 status and secondary hyperparathyroidism in relation to osteoporosis in elderly Swedish women. E-published ahead of print in *Osteoporosis International* 2007-09-14.

IV Salminen H, Sääf M, Johansson S-E, Ringertz H, Strender L-E
The effect of transvaginal estradiol on bone in aged women; a randomised controlled trial. *Maturitas* 2007; 57; 370–381.

CONTENTS

LIST OF ABBREVIATIONS

AUC	Area under curve
BMD	Bone mineral density (g/cm^2)
BMI	Body mass index
CEE	Conjugated equine estrogens
CI	Confidence interval
CV	Coefficient of variation
DXA	Dual X-ray absorptiometry
DXL	Dual X-ray and Laser absorptiometry
HERS	Heart and Estrogen/progestin Replacement Study
HRT	Hormone replacement therapy
IGF-I	Insulin-like growth factor I
IGFBP-1	Insulin-like growth factor binding protein 1
ISCD	International Society for Clinical Densitometry
MNA	Mini Nutritional Assessment
MPA	Medroxy-progesterone acetate
NHANES III	National Health and Nutrition Examination Survey III
OB	Osteoblasts
OC	Osteoclasts
OR	Odds ratio
PRIMOS	Primary Health Care and Osteoporosis project
PTH	Parathyroid hormone
RANKL	Receptor activator of nuclear factor-kB ligand
RCT	Randomised controlled trial
RECORD	Randomised Evaluation of Calcium or vitamin D
ROC	Receiver operator characteristics
RR	Relative risk
SD	Standard deviation
SE	Standard error
SHBG	Sex hormone binding globulin
T-score	Patient's BMD related to the mean BMD of a young normal healthy reference population
25(OH)D	25-hydroxy vitamin D
WHI	Women's Health Initiative Study
WHO	World Health Organisation
Z-score	Patient's BMD related to the mean BMD of an age-matched reference population

1 INTRODUCTION

Osteoporosis is a major risk factor for fracture and one of the major health problems in the world, considering the costs for the fragility fractures and the individual suffering. Both hip and vertebral fractures are associated with excess mortality (Kado, Browner et al. 1999; Johnell, Kanis et al. 2003). Sweden, Norway and Iceland have the highest incidence of osteoporotic fractures in the world (Johnell, Gullberg et al. 1992; Kanis, Johnell et al. 2002). Annually, approximately 18,000 hip fractures and about 25,000 wrist fractures occur in Sweden. Vertebral fractures often remain undiagnosed although they are very common (Cooper, Atkinson et al. 1992; Grigoryan, Guermazi et al. 2003; Grados, Marcelli et al. 2004; Kanis, Johnell et al. 2004). Elderly women are seldom evaluated despite a history of a previous fracture. Many of these women visit their family doctors at the primary health care centres for their other medical conditions but their osteoporosis is seldom diagnosed and treated. Osteoporosis is a common condition among elderly, the prevalence in Sweden rising from every fifth woman in the age of 60–69 years to about every third ten years later (Läkemedelsverket 2007). In white populations, every second woman over 50 suffers a fragility fracture in her remaining lifetime (Kanis and Gluer 2000). In fact, white women have a greater risk of hip fracture (one in six) compared with a one in nine risk of breast cancer (van Staa, Dennison et al. 2001). The studies presented in this thesis are part of the PRIMOS project (Primary Health Care and Osteoporosis), where we have studied different aspects of osteoporosis among elderly women in primary health care. The PRIMOS project has two parts, a cross-sectional study and a subsequent randomised controlled clinical trial. The cross-sectional part of the PRIMOS project studies the epidemiology and risk factors for osteoporosis and osteoporotic fractures among elderly women. It also includes methodological comparison of central bone mineral density measurements with peripheral measurements of the calcaneus. The second part of the PRIMOS project is a randomised controlled clinical trial where we study the effect of ultra-low doses of estradiol on bone mineral density.

1.1 OSTEOPOROSIS – DEFINITIONS

Roughly every ten years, the entire adult skeleton is replaced by remodelling. Osteoporosis is the result of an imbalance between bone resorption and bone formation. Osteoporosis occurs when there is an uncoupling between the bone formation of osteoblasts and bone resorption of osteoclasts, and bone resorption exceeds bone formation. This imbalance leads to increased fragility of bone. Osteoporotic fractures usually result from a combination of increased fragility of bone and increased rate of falls.

The first internationally accepted definition of osteoporosis came in 1993:

"A systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk."

This definition is of little help when we want to diagnose osteoporosis. Osteoporosis is a part of the human ageing process and we can divide osteoporosis into *primary osteoporosis* caused by natural ageing and other risk factors, and *secondary osteoporosis* caused by other medical conditions and their treatment.

The Dual X-ray Absorptiometry (DXA) gives us a possibility to measure bone mineral density (BMD) very accurately. Thus, it can be used as a diagnostic test for osteoporosis. The WHO definition of osteoporosis 1994 is based on bone mineral density. A cut-off value of –2.5 SD below the BMD of young adult mean value was chosen to define osteoporosis. The WHO definition was originally created mainly for epidemiological purposes but is commonly used today for clinical purposes. When the WHO definition was created the sites of measurement of bone mineral density included in the definition were the femoral neck, lumbar spine and forearm. The cut-off points below identify approximately 30% of postmenopausal women as osteoporotic. The definition only applies to postmenopausal women and not to younger women and not to men.

WHO definition 1994 (WHO 1994):
1. *Normal bone density,* BMD not more than 1 SD below the young adult mean BMD.
2. *Osteopenia*, BMD more than 1 SD below the young adult mean BMD but less than 2.5 SD below the young adult mean BMD.
3. *Osteoporosis,* BMD 2.5 SD or more below the young adult mean BMD.
Established (or severe) osteoporosis is defined as an osteoporotic BMD value in a patient with an osteoporotic fracture.

The diagnosis of osteoporosis is based on an osteoporotic BMD value. It is important to remember that the decision about treatment is a weighted judgement where BMD is one of the risk factors taken into account.

Measuring bone mineral density is the best available non-invasive method to assess bone strength in clinical practice, but we must remember that there are other skeletal factors, often called bone quality, contributing to bone strength such as bone shape, bone microarchitecture and bone turnover (Heaney 2003).

1.2 MEASUREMENT OF BONE MINERAL DENSITY

1.2.1 DXA technology

Bone mineral density is accurately measured with DXA (Dual X-ray Absorptiometry). X-rays with two different energies are passed through the bone, some of the energy is absorbed, and the rest is detected on the other side of the body. In every “picture element” two variables can be defined. Two energies allow an estimation of the content of soft tissue (which contains water and fat) separated from the bones. The greater the bone mineral content of the skeleton, the more energy is absorbed. This radiation energy is detected and converted into an areal density measured in g/cm^2. A problem with the DXA method is that we do not fully know how the soft tissue content outside the bone differs from the soft tissue inside the bone. The amount of fat inside the skeleton differs at different ages and increases in

elderly. This introduces an error in determining the amount of hydroxy apatite inside the bone, an error that can reach 10–20% (Bolotin, Sievanen et al. 2001).

Basically, the traditional DXA technology is a two component system but it can be used to determine the content of water, fat and bone in the body. This is done by determining the content of water and fat in all picture elements outside the bone and then extrapolating the same fat and water content to exist inside the bone, introducing a minor error in the estimation.

The DXA technology is most often used for measurements of the lumbar spine and the hip, but there are also DXA technologies for peripheral sites such as the radius and the calcaneus. Light portable devices have been developed for measuring BMD in the calcaneus. This equipment offers a possibility to perform measurements at the local primary health care centre. These devices do not occupy much space, are easy to handle and do not cost as much as the central DXA devices. Several previous studies have compared methods for measuring heel BMD with axial measurements (Greenspan, Bouxsein et al. 1997; Diessel, Fuerst et al. 2000; Fordham, Chinn et al. 2000; Williams and Daymond 2003). The studies have shown that calcaneal measurements of bone density with DXA technique can discriminate quite well between groups of osteoporotic subjects and those without the condition. Some of the studies have also proposed other cut-off points than –2.5 SD for the heel devices and have not found the WHO cut-off point to be applicable to the heel (Fordham, Chinn et al. 2000; Williams and Daymond 2003).

1.2.2 DXL technology

DXL stands for Dual X-ray and Laser technique. It was developed in Sweden by Kullenberg and co-workers. It combines the DXA technology with a laser beam measurement of the thickness of the object. Thus, in every “picture element”, three known variables can be measured (the absorption of the two X-ray energies and the thickness of the object) and three variables inside the bone can be determined: the content of hydroxy apatite, the amount of fat and the amount of water. DXL can be described as a three-component system. The DXL method is described by Hakulinen and colleagues (Hakulinen, Saarakkala et al. 2003).

The heel DXL devices have gained popularity in recent years in Sweden but have not been evaluated enough. In Läkartidningen, the journal of the Swedish Medical Association, there has been a discussion and also warnings from a group of experts on osteoporosis, about the widespread use of heel DXA measurements (Kullenberg 2002; Ljunggren 2002; Ersson 2003; Ljunggren, Mallmin et al. 2003). There is insufficient knowledge as to whether the same cut-off point of –2.5 SD is also appropriate for heel BMD measurements. Further, we do not know the fracture-prospective value of a calcaneal measurement. It cannot be used for monitoring treatment because there are very few studies evaluating the calcaneus response to osteoporosis treatment. Furthermore, using measurement of a peripheral site for diagnostic purpose, is not recommended by the experts of ISCD (International Society for Clinical Densitometry) (Leib, Lenchik et al. 2002).

1.2.3 T-scores and Z-scores

Values of bone mineral density are measured in g/cm^2 and then converted into *T*-scores and *Z*-scores. The origin of the *T*-score is described by Faulkner (2004).

T-scores are related to the young mean peak bone mass (the young normal healthy mean BMD) of the reference population of the same gender and are calculated according to the following formula:

$$T\text{-score} = \frac{\text{patient's BMD} - \text{young adult mean BMD of the reference population}}{\text{standard deviation (SD) of the young mean peak BMD}}$$

Z-scores are related to the mean BMD in the reference population with the same age group as the patient and are calculated according to the following formula:

$$Z\text{-score} = \frac{\text{patient's BMD} - \text{mean BMD of age-matched reference population}}{\text{standard deviation (SD) of the young mean peak BMD}}$$

T-scores are used for the densitometric diagnosis of osteoporosis:
1. Normal: a *T*-score ≥ -1
2. Osteopenia: *T*-score < -1 and > -2.5
3. Osteoporosis: *T*-score ≤ -2.5
4. Established or severe osteoporosis: *T*-score≤ -2.5 and the presence of one or more fragility fractures.

1.2.4 Reference populations

The influence of the chosen reference population on *T*- and *Z*-scores is substantial (Chen, Maricic et al. 1998; Faulkner, von Stetten et al. 1999). Ideally, the reference population should be of the same ethnicity and same gender and the sample should reflect the underlying population.

As yet, we have no ideal Swedish reference population of our own to use when we measure the hip and lumbar spine. In the study by Löfman and co-workers it was shown that Swedish women have very similar BMD values to the North American Caucasian population measured in the NHANES III survey (Lofman, Larsson et al. 1997). *T*-scores based on this Swedish population, differ a lot from those based on the NHANES III reference population, probably because the peak bone mass was based on a small sample of only 27 individuals (peak bone mass femoral neck 0.81g/cm^2 (SD 0.11).

The reference populations used in this work are:

For lumbar spine:
The Hologic original; the only reference population available for the Hologic equipment for lumbar spine is the original consisting of 605 North American women with unspecified ethnicity. The reference population is not described in any published article.

For hip:
1. The Hologic original; the reference population is not described in any published article. It consists of 747 women for the proximal femur.
2. NHANES III; the third National Health and Nutrition Examination Study. The reference population consists of 1541 Caucasian women (special reference

populations were created for Non-Hispanic Blacks and Mexican Americans; males have their own reference values). The peak bone mass of 0.849 g/cm^2 (SD 0.109) was based on 194 measurements in women aged 20–29 years (Looker, Wahner et al. 1995).

For calcaneus:
1. The original reference population; it consists of 401 Swedish women. The peak bone mass was based on measurements of 27 women (aged 18–22 years) The peak bone mass was 0.512 g/cm^2 (SD 0.08). The reference population is not described in any published article.
2. The Calscan database; this consists of 993 Swedish women with a peak bone mass derived from the age interval 18–27 years with the highest bone mass at 22 years of age. The peak bone mass was 0.483 g/cm^2 (SD 0.062) (Kullenberg 2003).

1.3 MAIN RISK FACTORS FOR OSTEOPOROSIS AND OSTEOPOROTIC FRACTURES

1.3.1 Age

Advanced age is the strongest individual risk factor for fragility fracture, demonstrated in many studies (Bauer, Browner et al. 1993; Ballard, Purdie et al. 1998; Siris, Miller et al. 2001). The highest bone mass, peak bone mass, is reached in young adults in both sexes 20–30 years of age (Bonjour, Theintz et al. 1991; Recker, Davies et al. 1992). For men, the decrease in bone mass is slow and continuous throughout life, while for women, the estrogen deficiency initiated by menopause accelerates the bone losses between age 50 and 60. Thereafter, the decrease is slower, resembling that of men but even between 60 and 80, women had greater bone losses than men, 19% in women compared with 10% in men in the study by Nguyen and colleagues (Nguyen, Kelly et al. 1994). The rate of bone losses in the first ten postmenopausal years varies from 1–5% per year (Hansen, Overgaard et al. 1991). Peak bone mass of women is not as high as that of men (Looker, Wahner et al. 1995) and when bone losses accelerate at menopause, the risk of fragility fracture increases rapidly. The most common first fragility fracture is the wrist fracture followed by vertebral compression fractures and hip fractures. The mean age for a woman to get her hip fracture is 81 years while that for men is 86 years, and therefore many men may already have died of other causes.

1.3.2 Anthropometric factors

BMI below 19–20 in elderly is often associated with osteoporosis while individuals with a weight over 70 kg are seldom affected (Michaelsson, Bergstrom et al. 1996). Low body weight and low body mass index (BMI) have consistently been shown to be associated with an increased risk of osteoporosis (Brot, Jensen et al. 1997; Meyer, Tverdal et al. 1998; Nguyen, Sambrook et al. 1998; Dargent-Molina, Poitiers et al. 2000). In the age category of the subjects in our study, low body weight rather than low BMI has the strongest association with osteoporosis. This may due to the fact that many individuals lose height due to the deformities in spine, and perhaps also due to vertebral fractures, often undiagnosed (Cooper, Atkinson et al. 1992; O'Neill,

Felsenberg et al. 1996). A reduction in height over 4–5 cm may be caused by vertebral fractures (Kantor, Ossa et al. 2004).

Also tall individuals have an increased risk of some osteoporotic fractures (Meyer, Tverdal et al. 1993). Weight variability and weight change are also risk factors for hip fractures (Melton, Therneau et al. 1998).

In our study we also study the association of the mid-arm circumference and the calf circumference with osteoporosis.

1.3.3 Low bone mineral density

One of the main risk factors for osteoporotic fractures is low bone mineral density, as has been shown in several studies (Cummings, Black et al. 1990; Cummings, Black et al. 1993; Gardsell, Johnell et al. 1993; Cheng, Suominen et al. 1997). These studies have established that both axial and peripheral bone density predict fractures in elderly women. A one SD decrease in bone mineral density at all measured sites (proximal and distal radius, hip, lumbar spine and calcaneus) was shown to have a predictive ability for future osteoporotic fracture with a pooled RR of 1.5 in a meta-analysis by the Swedish Council of Technology Assessment in Health Care (Marshall, Johnell et al. 1996). In this meta-analysis calcaneus had a predictive ability of RR 2.0 for each SD decline of BMD for future hip fracture and a slightly higher predictive ability than the lumbar spine to predict a future vertebral fracture (RR 2.4 and 2.3 respectively). Measurement of BMD at the site of the future fracture region is considered to have the best predictive ability. In the meta-analysis above, the ability of the BMD measurement of the hip to predict a future hip fracture showed a RR of 2.6 for a decline of 1 SD in BMD. In another meta-analysis by Cummings and co-workers the calcaneal BMD was found to have a RR of 1.8 for a decline of 1 SD in BMD to predict future hip fracture (Cummings and Melton 2002).

Despite the fact that low BMD is a major risk factor for osteoporotic fracture, over 80% of the fractures in postmenopausal women occur in those women who did not have a peripheral measurement showing osteoporosis (Siris, Chen et al. 2004). It is also important to remember that the decision about treatment is based not only on a BMD value showing osteoporosis. All individuals with low BMD values do not require treatment and sometimes, especially in the presence of a fragility fracture or treatment with corticosteroids, BMD values above the cut-off point of osteoporosis do not exclude the need for bone specific agents.

1.3.4 Previous fracture

Epidemiological studies have shown that a previous fragility fracture is a major risk factor for subsequent fractures (Bauer, Browner et al. 1993; Lauritzen and Lund 1993; Honkanen, Tuppurainen et al. 1997; Gunnes, Mellstrom et al. 1998; Lindsay, Silverman et al. 2001; Siris, Miller et al. 2001; van Staa, Leufkens et al. 2002). The most common sites of fragility fractures are the radius, humerus, vertebra and hip. The lifetime risk of a hip fracture for a Swedish middle-aged woman is 23% (Kanis, Johnell et al. 2000). The combination of decreased bone mineral density and several risk factors leads to a greatly elevated risk of fracture, as shown in several major studies (Cummings, Nevitt et al. 1995; Johnell, Gullberg et al. 1995; Siris, Miller et al. 2001). Those with most risk factors in combination with low bone density had up

to 25-fold higher risk of fracture as shown by Cummings (Cummings, Nevitt et al. 1995).

1.3.5 Propensity to fall

The osteoporotic skeleton is like a fragile vase, it does not break until it is tipped over. For more osteoporotic fractures with the exception of vertebral compression fractures, which may occur spontaneously, some kind of stress, mostly a fall, is required in order to incur a fracture. The rate of falls increases with age (Blake, Morgan et al. 1988; Sinaki 2004; Vu, Weintraub et al. 2005), and the skeleton gets more and more fragile, thus creating a high risk combination. The origin of these falls is multifactorial: impaired balance, impaired vision, some medications, weaker muscles and the degenerative changes contributing.

In the report on osteoporosis from the Swedish Council on Technology Assessment in Health Care 2003, the working group concludes that individual training of muscle strength and balance have proved to decrease the risk of falls (SBU 2003).

In a randomised controlled trial by Bischoff-Ferrari and colleagues, vitamin D was shown to reduce the odds of falling by 46% in ambulatory older women during a three-year supplementation with 500 mg calcium and 700 IU of vitamin D (Bischoff-Ferrari, Orav et al. 2006).

A recent randomised trial of Broe and colleagues showed in a group of 124 nursing home residents (average age 89 years), who were randomly assigned to receive one of four vitamin D supplement doses (200 IU, 400 IU, 600 IU, or 800 IU) or placebo daily for 5 months, that participants in the 800 IU group had a 72% lower adjusted-incidence rate ratio of falls than those taking placebo over the 5 months of the trial (Broe, Chen et al. 2007).

1.3.6 Other risk factors

Smoking
Smoking is a strong risk factor which doubles the risk of osteoporosis, as shown in the meta-analysis by Kanis and colleagues (Kanis, Johnell et al. 2005).

Heredity and ethnicity
Bone mineral density may by determined by genetics up to about 70% (Flicker, Hopper et al. 1995). The risk of osteoporotic fracture is doubled if the patient's mother has suffered a hip fracture (Cummings, Nevitt et al. 1995). In a meta-analysis Kanis and colleagues showed that a family history of hip fracture in parents was associated with a significant risk increase of about 50% of all osteoporotic fracture (RR 1.54; 95CI=1.25–1.88) and of hip fracture (RR=2.27; 95% CI=1.47–3.49) (Kanis, Johansson et al. 2004).

The risk of fragility fractures varies considerably around the world. Sweden is one the countries where the 10-year risk of hip fracture is highest; only Norway and Iceland have higher risks (Kanis, Johnell et al. 2002).

Female gender
The lifetime risk of a 50-year-old Swedish woman suffering from an osteoporosis-related fracture is 50%, compared with the lifetime risk of a Swedish man of the same age which is 25% (Kanis, Johnell et al. 2000).

Other risk factors include *menopause before the age of 45 years, physical inactivity* and *high consumption of alcohol* (SBU 2003).

Multiple risk factors in one individual increase the individual risk very rapidly, as shown by Cummings and colleagues in the SOF (Study of Osteoporosis Fractures) study (Cummings, Nevitt et al. 1995). An international working group has been trying to develop an algorithm for the risk calculation for many years and hopefully we can soon have better risk estimates for the individual risk than we have had previously to support us in our clinical practice (Kanis, Oden et al. 2007). One big step was the calculation of absolute 10-year risks of fracture (Kanis, Johnell et al. 2001).

1.4 CALCIUM REGULATING HORMONES

Regulation of calcium-phosphate homeostasis and bone remodelling is controlled by interactions between parathyroid hormone (PTH) and vitamin D.

1.4.1 Parathyroid hormone (PTH)

Parathyroid hormone (PTH) is produced by the chief cells of the parathyroid glands. PTH has a major role in the calcium homeostasis and exerts its main effects in bone and kidney. The main regulators of PTH secretion are the extracellular concentrations of calcium and phosphate and of 1,25(OH_2)D. PTH stimulates the release of calcium and phosphate from bone. PTH acts directly on the osteoblast lineage cells, influencing their differentiation and function but is also involved in osteoclast differentiation and activation. Osteoblasts express RANKL (receptor activator of the nuclear factor kB ligand) on their surface. Calciotropic hormones and growth factors, among others, affect the expression of RANKL. PTH-induced bone resorption and calcium mobilisation take place by altering the expression of RANKL on the osteoblast surface (Blair, Zheng et al. 2007). The binding of PTH by osteoblast receptors promotes the activation of RANKL on the RANKL receptor present on the precursors and mature osteoclasts, an effect that may be partly mediated by IGF-I. In kidney, PTH increases the reabsorption of calcium and inhibits that of phosphate. PTH also increases the activity of the renal hydroxylation of 25(OH)D and in that way increases the intestinal absorption of calcium and phosphate.

The most important regulators of the secretion of PTH are the extracellular CaSR (calcium sensing) receptors.

The parathyroid chief cells have VDR (vitamin D receptor), which responds to the active form of vitamin D, 1,25$(OH)_2$D, by decreasing the expression of the PTH gene and decreasing the synthesis and secretion of PTH. When the stores of vitamin D of the body are depleted, the efficiency of intestinal calcium absorption decreases, affecting serum calcium concentrations. Decreased serum calcium leads to increased secretion and synthesis of PTH (Holick 2003; Dawson-Hughes, Heaney et al. 2005).

The overall result is a state of secondary hyperparathyroidism with normal serum calcium, levels, elevated levels of PTH, elevated levels of alkaline phosphatase and low or normal serum phosphorous. The mineralisation of the bone is affected, in severe cases osteomalacia (Holick 2003).

1.4.2 Vitamin D

Vitamin D is a steroid hormone and its effects on bone are mediated through the active form $1,25(OH)_2D$. Its main effect is to maintain serum calcium levels within normal limits. It accomplishes its effect by increasing calcium absorption from the intestines and by facilitating osteoclast precursors to become mature osteoclasts, which in turn mobilises calcium from the skeleton into circulation (Holick 2003). $1,25(OH)_2D$ acts in similar manner to other steroid hormones and has its own VDR (vitamin D receptors) in the cytoplasm initiating a cascade of reactions, finally resulting in the transcription of the vitamin D responsive gene (Haussler, Whitfield et al. 1998). The keeping of serum calcium within a narrow window is important for its function in a wide variety of metabolic functions, signal transduction and in neuromuscular activity (Holick 2003). With increasing age, there is a decrease in the ability of our skin to build precursors of the active vitamin D in the skin when exposed to sun. At the same time, the ability of our kidneys to activate 25(OH)D decreases. In the gut, both calcium and phosphorous absorption from food is enhanced. With adequate vitamin D concentrations, 30–40% of dietary calcium is absorbed, this absorption covering the daily losses via kidneys of about 160–200 mg.

Several studies have shown that a combination of calcium and vitamin D3 has a fracture-preventive effect, best shown in institutionalised women (Dawson-Hughes, Harris et al. 1997; Chapuy, Pamphile et al. 2002; Papadimitropoulos, Wells et al. 2002). In the meta-analysis by Bischoff-Ferrari and colleagues in 2005 of controlled randomised trials, vitamin D supplementation in doses 700–800 IU/day lowered the risk of hip fracture by 26% and the risk of non-vertebral fracture by 23% (Bischoff-Ferrari, Willett et al. 2005).

Three large clinical trials, the RECORD trial, the WHI trial, and the Porthouse trial, have not shown any fracture-preventive effect of calcium and vitamin D therapy (Grant, Avenell et al. 2005; Porthouse, Cockayne et al. 2005; Jackson, LaCroix et al. 2006). Both the RECORD trial and the Porthouse trial had treatment doses of 800 IU of vitamin D3 but both trials had a low compliance (over 50% of the participants in the RECORD trial had stopped taking their pills after 24 months and 40% had ceased the medication after 12 months in the Porthouse trial). The WHI trial is a large randomised trial with over 36,000 participants with a mean age of 62 years randomised either to calcium 1000 mg and vitamin D3 400 IU or to placebo, but many of the subjects were already on calcium and vitamin D supplementation and were also randomised to therapy with estrogen (Jackson, LaCroix et al. 2006). A Cochrane report from 2005 showed that there is strong evidence for the fracture-preventing effect of calcium and vitamin D in combination in elderly institutionalised women, but that the effect is more uncertain for younger free-living women (Avenell, Gillespie et al. 2005). A recent meta-analysis of 17 trials with fracture as an outcome by Tang and colleagues reported a 12% risk reduction in fractures of all tyrpes (Tang, Eslick et al 2007). The treatment effect was better with calcium doses of 1200 mg or more and with vitamin D3 doses of 800 IU or more than doses less than 800 IU.

Vitamin D is believed be important for the muscle strength of elderly people. In a Swedish study of 969 women, 25(OH)D levels correlated with physical activity, the Romberg balance test, gait speed and thigh muscle strength (Gerdhem, Ringsberg et al. 2005). Bischoff-Ferrari and colleagues found higher serum 25(OH)D levels to be associated with better lower extremity function (Bischoff-Ferrari, Dietrich et al. 2004).

In the Longitudinal Aging Study Amsterdam, low 25(OH)D levels <25 nmol/L were approximately twice as likely to experience sarcopenia (based on muscle mass and grip strength) after a follow-up of three years, as persons with higher (>50 nmol/L) levels of vitamin D (Visser, Deeg et al. 2003).

1.5 ESTROGENS

Already in 1947, Fuller Albright showed how estrogens had a positive effect on the calcium losses of women with osteoporosis-related fractures (Albright F 1940; Albright 1947). Albright also noticed how height loss was unusual in estrogen-treated patients.

Steroid hormones can be divided into three categories: glucocorticoids, mineralocorticoids and gonadal steroids. Estrogen is one of the gonadal steroids, the others are androgens and progesterone. The steroid hormones are synthesised from cholesterol. Through pathways involving about 10 enzymes, estrogen is finally formed from testosterone. This reaction is mediated by the enzyme aromatase. Aromatase is present in ovaries and also in adipocytes and osteoblasts and osteoclasts in bone.

The major form of estrogen in postmenopausal women is estrone, one of the two steroids formed from androstenedione, the other steroid being testosterone. Most of the estrogen in circulation is bound to a specific carrier protein SHBG (sex hormone binding globulin) and only approximately 1–3% of the estrogen is free in circulation; 30–50% is bound to albumin and the rest to SHBG. Both the free and albumin-bound fraction can enter cells by diffusion through the cell membrane (Seibel M 1999).

Estrogens have special receptors and these receptors have been found in both human osteoblasts and osteoclasts. Estrogens reduce bone resorption by inhibiting osteoclastogenesis and the function of osteoclasts.

Estrogen deficiency is the most important cause of postmenopausal bone loss in women. Women lose annually in average 3% of their bone mass in the years after menopause, compared with men who only lose 0.5–1% of their bone mass per year during the same period (Guthrie, Dennerstein et al. 2000). Estrogens are believed to play an important role for women as well as for men in the maintenance of bone mass (Riggs, Khosla et al. 1998; Khosla, Melton et al. 2001; Khosla 2004). Endogenous estrogens have an important role for bone health even after menopause. Cummings and colleagues showed in 1998, in a population-based study of women over 65 years of age, that women with estradiol levels lower than 5 pg/ml had lower bone mass than women who had higher estrogen levels, and that these women also had a higher risk of vertebral and hip fractures (Cummings, Browner et al. 1998).

The attitude towards treatment with estrogens has changed dramatically in recent years. This is the result of two large-scale randomised controlled trials with estrogen, the HERS (Heart and Estrogen/progestin replacement study) and the WHI (Women's Health Initiative) trial (Hulley, Grady et al. 1998; Rossouw, Anderson et al. 2002). HERS is the only large study where treatment with estrogen/progestin showed no fracture prevention, while WHI confirmed the results from epidemiological studies and minor RCTs and showed a risk reduction for hip fracture of 33%. This fracture reduction is an impressive achievement taking into account that the study population of WHI was quite young (mean age 62 years) and healthy. What HERS showed was a negative total risk benefit balance for treatment with estrogen/progestin in doses of

0.625 mg CEE (conjugated equine estrogen) with the addition of MPA (medroxy-progesterone acetate). The cardiovascular risk was increased during the first year of treatment and the risk of breast cancer and venous thromboembolism was increased. The risk of colon cancer was reduced. The increased risk of breast cancer may be mostly correlated to the combined use of progestin and estrogen and not estrogen treatment alone (Li, Malone et al. 2003; Stefanick, Anderson et al. 2006; Rossouw, Prentice et al. 2007). Estrogen may still have some place in the treatment arsenal for early post-menopausal women. For women under 60 years of age, the cardiovascular risk profile seems more favourable. A recent meta-analysis of 23 randomised studies with a total of nearly 40,000 women and more than 190,000 person-years of follow-up showed a risk reduction of about 40% for women who started their HRT when they were under 60 years of age (Salpeter, Walsh et al. 2006). So, in the early postmenopausal population, the risk benefit equation may be quite different from that seen in HERS and WHI trials, especially among those who are hysterectomised and do not need the addition of progesterone to counteract endometrial hyperplasia. Also, the risk benefit balance could be different when lower doses are administered and other forms of estrogen than CEE are used.

Estrogen may also have beneficial effects besides the fracture-preventive effect and the risk reduction for colon cancer. A meta-analysis of 107 RCTs with over 30,000 women showed that treatment with estrogen reduces the abdominal fat, the insulin resistance and enhances the lipid profile among women without diabetes. In diabetic women the insulin resistance and the levels of fasting blood glucose were affected in a positive direction (Salpeter, Walsh et al. 2006).

The fracture-preventive effect of estrogens may partly be mediated through a positive effect on postural balance (Naessen, Lindmark et al. 1997; Naessen, Lindmark et al. 2007).

The fracture-preventive effect of estrogens in traditional doses is well established both through epidemiological studies and RCTs (Nachtigall, Nachtigall et al. 1979; Lufkin, Wahner et al. 1992; Cauley, Seeley et al. 1995; Rossouw, Anderson et al. 2002). During the last ten years more and more evidence has also been gathered of a beneficial role of lower doses of estrogens. Lindsay and colleagues showed in 2002 that estrogen administered in the form of CEE with or without the addition of progestin had favourable effects such as reduced bone remodelling. The biochemical markers were only slightly less suppressed with the dose of 0.3 mg of CEE compared with the traditional dose of 0.625 mg of CEE (Lindsay, Gallagher et al. 2002). Prestwood and colleagues showed in a randomised controlled trial in 2000 that a low dose of 0.25 mg micronised 17 beta-estradiol had a positive effect on bone turnover in elderly women (Prestwood, Kenny et al. 2000). A gain of bone mass could also be shown with an ultra-low dose of 0.014 mg estradiol transdermally (Ettinger, Ensrud et al. 2004). Already in 1997, in a Swedish pilot study by Naessen and colleagues, a small dose of 0.0075 mg per day administered through a vaginal ring increased the BMD of ultradistal radius by about 2% after a follow-up of 6 months (Naessen, Berglund et al. 1997).

1.6 IGFS AND IGFBPS

Growth hormone (GH) and insulin-like growth factors (IGFs) play an important role in the development and growth of the skeleton, and the GH/IGF-I axis also has an important role for maintaining bone mass (Rosen 2004; Niu and Rosen 2005). GH stimulates the release of the IGFs from the liver and the IGFs have endocrine effects. But IGFs are also secreted from many tissues such as bone and adipose tissue, for paracrine and autocrine effects to regulate bone resorption and/or formation (Govoni, Baylink et al. 2005; Govoni, Amaar et al. 2006). Bone is a major reservoir for IGF-I and IGF-II. IGF-II is the quantitatively dominant. IGF-II is not regulated by growth hormone in contrast to IGF-I. There has been a lack of methods to determine levels of IGF-II accurately, furthermore IGF-II levels in circulation are not believed to reflect levels in bone tissue. Nutritional factors are believed to be determinants of both IGF-I and IGF-II levels. The IGFs increase bone formation by regulating the proliferation, differentiation and apoptosis of osteoblasts (Rosen and Donahue 1998; Mohan, Richman et al. 2003).

Serum levels of GH and IGF-I decrease with age. The decrease of IGF-I with age could be partly due to lower protein intake in the elderly, as protein intake is one of the major determinants of IGF-I (Bonjour, Schurch et al. 1997). Low levels of IGF-I may increase the risk of fractures, as shown in a French longitudinal study where low levels of IGF-I predicted osteoporotic fractures independently of the BMD (Gamero, Sornay-Rendu et al. 2000). In two recent Japanese studies among women with diabetes mellitus type 2, it was shown that IGF-I could identify women with vertebral fractures while their lumbar BMD was not associated with the presence of vertebral fractures (Kanazawa, Yamaguchi et al. 2007; Yamamoto M 2007). Hip fracture patients have lower levels of IGF-I (Hedstrom 1999; Ponzer, Tidermark et al. 1999; Campillo, Paillaud et al. 2000). Protein supplementation for recent hip fracture patients increased the levels of IGF-I and shortened the rehabilitation in patient care time by 25% in a randomised controlled trial with 82 patients with a duration of six months (Schurch, Rizzoli et al. 1998).

Several previous studies have shown correlations between BMD and IGF system components. In the study by Boonen, IGF-I was found to be an independent predictor of BMD at all femoral sites (Boonen, Lesaffre et al. 1996). In the study by Szulc and colleagues, IGF-I was found to be correlated with total hip BMD in men aged 19–60 years (Szulc, Joly-Pharaboz et al. 2004). In the study by Yamaguchi and colleagues, IGF-I was positively correlated to BMD at all sites measured except the femoral neck (Yamaguchi, Kanatani et al. 2006). In the same study, the levels of serum IGF-I, IGFBP-3 and IGFBP-5 were significantly lower in women with vertebral fractures than those without fractures. Krassas and colleagues have reported a positive correlation between BMD and the IGF-I and IGFBP-3 levels (Krassas, Papadopoulou et al. 2003). In the study by Gillberg among Swedish men the levels of IGF-I and IGFBP-3 were positively correlated with femoral neck BMD (Gillberg, Olofsson et al. 2002). In the Framingham heart study, IGF-I was positively associated with BMD at all sites (hip, lumbar spine and radius) in women but not in men (Langlois, Rosen et al. 1998). In the Rancho Bernardo Study, Jassal and colleagues found a correlation between IGFBP-1 and BMD at the spine and hip but, after adjustment for age and BMI, there remained no significant association between IGFBP-1 and BMD (Jassal, von Muhlen et al. 2005).

About 97–99% of the circulating IGF-I is bound to six binding proteins IGFBP-1–6. The carrier proteins control and modify both the endocrine and local actions of the IGFs but may also have their own effects on the skeleton. Their independent roles affect cell migration, cell growth and apoptosis (Mohan, Richman et al. 2003). These binding proteins are mainly produced by the osteoblasts and modulate the action of IGF-I in both positive and negative manner. IGFBP-1, -2 -4 and -6 inhibit and IGFBP-3 and -5 stimulate osteoblast function. In patients with osteoporosis and fractures, the inhibitory IGFBPs are increased and the stimulatory IGFBPs decreased (Jehle, Schulten et al. 2003). In this case-control study by Jehle and colleagues, osteoporosis patients had a 73% decrease in IGF-I, a 10% decrease in IGFBP-3 and a 52% decrease in IGFBP-5. At the same time, their levels of IGFBP-1 were increased fourfold, IGFBP-2 levels were 80% higher, IGFBP-4 30% higher and IGFBP-6 increased twofold compared with the age- and sex-matched control subjects.

IGFBP-1 can be produced by osteoblast-like cells and can inhibit IGF-I binding to its receptors. IGFBP-1 inhibits collagen gene expression by blocking the effect of IGF-I. The IGFBPs can be associated with cell surfaces and are regulated post-translationally by phosphorylation and glycosylation. In humans, phosphorylated IGFBP-1 has a sixfold greater affinity for IGF-I than non-phosphorylated IGFBP-1 (Conover 1996; Firth, McDougall et al. 2002). The IGF-independent actions of IGFBP-1 are due to its binding to a specific receptor, alpha 5 beta 1 receptor (Gleeson, Chakraborty et al. 2001).

IGFBP-2 has been found to be the most predictive of increased bone turnover (Amin S 2007) and the one most correlated to levels of bone resorption markers. Higher serum IGFBP-2 is also a predictor of lower BMD.

The dominant binding protein in circulation is IGFBP-3, which binds 80% of the IGF-I (Firth and Baxter 2002). Serum levels of IGFBP-3 decrease with age while those of IGFBP-1 increase with advancing age. IGFBP-3 levels are positively regulated by GH and/or IGF-I.

The IGFBP-4 and -5 are the most abundant IGFBPs produced by the osteoblasts. IGFBP-4 inhibits osteoblast function by sequestering IGF and preventing its binding to its receptor (Mohan and Baylink 1996; Mazerbourg, Callebaut et al. 2004; Govoni, Baylink et al. 2005). IGFBP-5 is a stimulator of bone formation. It associates with proteins on the cell surface and increases local concentrations of IGFs in the vicinity of IGF receptors (Mohan and Baylink 1996; Mohan and Baylink 2002). IGFBP-5 has a higher affinity to IGF-II than to IGF-I and regulates the storage of IGF-II in bone. The actions of IGFBP-5 are regulated by proteases capable of fragmenting IGFBP-5.

IGFBP-6 has a greater affinity for IGF-II than for IGF-I and is mainly inhibitory (Gabbitas and Canalis 1997; Jehle, Schulten et al. 2003).

1.7 NUTRITION

1.7.1 Malnutrition in elderly – how to define?

There is no consensus on how to define malnutrition among elderly and no exact definition of the condition. Many methods have been developed but we still lack a

gold standard to define malnutrition and protein-energy malnutrition (PEM) (Akner and Cederholm 2001).

Many nutritional markers such as serum albumin and IGF-I have shown to be correlated with clinical malnutrition (Omran and Salem 2002). Serum albumin levels are a good predictor of survival or death after fracture (Rico, Revilla et al. 1992).

Many different kinds of assessment tests have been introduced. In a review of over 40 different instruments Jones and colleagues found in 2002 that many of the instruments were poorly validated (Jones 2002). The expert group from Espen (European Society for Clinical Nutrition and Metabolism) have stated in their guidelines (Kondrup, Allison et al. 2003) that the MNA instrument is one of the three best suited to assess the nutritional status of the elderly. Three instruments are described for screening of malnutrition among adults: MNA, MUST (Malnutrition Universal Screening Tool) and NRS-2002 (Nutritional Risk Screening). Another instrument that is commonly used especially among surgical patients is SGA (Subjective Global Assessment) (Guigoz and Vellas 1997; Todorovic 2001; Kondrup, Rasmussen et al. 2003).

1.7.2 Protein

Bone remodelling requires an input of dietary protein because many of components in bone matrix containing protein cannot be recycled. Bone loss has been found to be inversely related to protein intake (Hannan, Tucker et al. 2000). Hip fracture patients often suffer from malnutrition and protein supplementation of hip fracture patients has been shown to improve outcome (Delmi, Rapin et al. 1990).

Protein energy malnutrition (PEM)
Protein is important for the growing skeleton and for optimal peak bone mass (Bonjour, Ammann et al. 2001). In a recent cross-sectional Danish study among 109 17-year-old boys and girls, a positive association was found between milk protein intake and size-adjusted bone mineral content, which remained significant even after adjustment for energy, calcium and physical activity (Budek, Hoppe et al. 2007). This association seemed not to be mediated by IGF-I because there was no significant association between IGF-I and milk protein intake. Loss of weight and reduced bone mineral density are often part of the ageing process. Protein-energy malnutrition leads to catabolism and to osteoporosis. PEM is related to reduced amounts of muscle mass and subcutaneous fat. The reduced muscle mass combined with osteoporosis also leads to increased propensity for falls (Sinaki, Brey et al. 2004). A Swedish study by Ponzer and co-workers has shown that half of the elderly with hip fractures had signs of PEM (Ponzer, Tidermark et al. 1999). In the randomised trial by Rizzoli and co-workers, it was shown that a protein supplementation for osteoporotic patients had positive effects and fewer new vertebral fractures were observed (Schurch, Rizzoli et al. 1998). Some studies have also shown decreased risk of fracture and bone density losses with increased intake of protein (Munger, Cerhan et al. 1999; Hannan, Tucker et al. 2000; Dawson-Hughes and Harris 2002; Promislow, Goodman-Gruen et al. 2002; Wengreen, Munger et al. 2004). In a meta-analysis by Hedström and colleagues in 2006, 19 randomised studies were identified where patients with hip fracture were treated with nutritional or anabolic treatment, 12 of them using nutritional or protein supplementation. Six of the studies showed improved clinical outcome with shorter

recovery period in hospital and fewer complications (Hedstrom, Ljungqvist et al. 2006).

1.7.3 Calcium

The human skeleton contains approximately 1 kg of calcium (for a male of 70 kg) and is the principal mineral of the skeleton. About 150–200 mg of calcium is absorbed from the intestines and the same amount of calcium is excreted mainly via urine every day. Calcium is an important element of the hydroxy apatite, needed to mineralise the skeleton. Calcium and vitamin D are also needed to prevent secondary hyperparathyroidism in the elderly (Chapuy and Meunier 1996). Whenever the absorbed calcium intake does not meet the demands and the losses, increased bone remodelling will be stimulated by PTH to keep balance in the extracellular fluid calcium ion homeostasis. When the calcium intake is appropriate for the demands, the PTH-stimulated remodelling increases immediately (Wastney, Martin et al. 2000).

A sufficient calcium intake around 1000 mg per day lowers the bone remodelling rate by 10–20% (Elders, Netelenbos et al. 1991). Calcium supplementation reduces both bone loss and tends to affect fracture rate in elderly as shown in the meta-analysis by Shea in 2002 (Shea, Wells et al. 2002).

Some widely used drugs affect the renal reabsorption of calcium, thiazides reduce it and loop diuretics increase the renal calcium losses.

In a meta-analysis of 31 trials, hormone replacement therapy was found to have greater increases in BMD when combined with calcium than when used without calcium supplementation (Nieves, Komar et al. 1998).

1.7.4 Vitamin D

Vitamin D is built under the influence of ultraviolet B rays on skin. This precursor, cholecalciferol or vitamin D3, is then activated in two steps, first through a 25-hydroxulation in the liver to 25(OH)D (calcidiol), followed by a 1-alpha-hydroxylation in the kidney to the active metabolite 1,25(OH)D (calcitriol). The activation in the kidneys is strictly regulated via PTH and the serum concentration of calcium and phosphate among others. Vitamin D is needed for normal mineralisation of the skeleton and a vitamin D deficiency during childhood and adolescence leads to rickets and to osteomalacia during adulthood. Active vitamin D increases the uptake of calcium from the gut.

Another source of vitamin D besides the sun is fat fish such as salmon and eel and fat dairy products. Fat fish contains approximately 400 IU vitamin D3 per 100 g. Milk in Sweden, but not in all countries in Europe, is fortified with vitamin D; 0.38 µg per dl (1 µg is 40 IU) of vitamin D3 is added to medium fat and light milk in Sweden. Standard milk (3% fat) is not fortified with vitamin D in Sweden; 1 dl of standard milk contains approximately 0.02 µg of vitamin D3. Margarine is fortified with 0.78 µg per 100 g.

The daily recommended intakes of vitamin D3 may be too low in most western countries (Vieth, Bischoff-Ferrari et al. 2007). The current Swedish recommended daily intake RDI of vitamin D3 for elderly people over 70 years is 400 IU (10 µg) per day. An increasing evidence shows that as much as 1000 IU (20 µg) may be needed (Bischoff-Ferrari, Willett et al. 2005; Hollis 2005; Mosekilde 2005; Vieth, Bischoff-Ferrari et al. 2007).

The best indicator of vitamin D status is the concentration of the metabolite 25(OH)D. 25(OH)D is formed after the first hydroxylation step in liver, a hydroxylation that is not rate-limited like the second hydroxylation in kidneys. This allows the circulating levels of 25(OH)D to reflect the amount of vitamin D3 absorbed or synthesised under the influence of the sun (Holick 2003; Hollis 2005). 25(OH)D is also the dominant form of vitamin D in circulation. The 25(OH)D levels decline with ageing; less vitamin D3 is formed in the skin because the amount of the precursor 7-dehydrocholesterol decreases and the elderly persons spend less time outdoors (MacLaughlin and Holick 1985). 25(OH)D concentrations show a seasonal variation and the concentrations are lowest during winter in Northern Europe. Elderly persons and especially those who live in institutions are at risk of vitamin D deficiency (Toss and Sorbo 1986; Lips, Hosking et al. 2006). In the study by Melin and colleagues, relatively few of the free-living elderly women were found have vitamin D deficiency (Melin, Wilske et al. 1999). Many immigrant groups in Scandinavia from countries in Middle East have vitamin D deficiency (Holvik, Meyer et al. 2005). In a recent study by Hyppönen and colleagues, the vitamin D status was surprisingly low in a large part of the British adult population. The levels were lowest during the winter and spring, when 25(OH)D concentrations <25, <40, and <75 nmol/L were found in 15.5%, 46.6%, and 87.1% in a birth cohort from 1958 with 7437 participants (Hypponen and Power 2007).

The use of a sunscreen with a sun protection factor 8 reduces the production of vitamin D3 by 95% (Holick, Matsuoka et al. 1995).

1.8 SECONDARY OSTEOPOROSIS

1.8.1 Osteoporosis due to treatment with some drugs

Corticosteroids

Oral treatment with corticosteroids is the most common cause of secondary osteoporosis. The mechanisms are an inhibition of bone formation, an increase of bone resorption and a decreased absorption of calcium from the gut. There is no safe lower dose, and also large doses of inhaled steroids may increase the risk of osteoporosis. Systemic treatment with corticosteroids > 3 months is one of the strong risk factors in the recent analysis from the Swedish Medical Drugs Agency (2007) and the report on osteoporosis in 2003 from the Swedish Council on Technology Assessment in Health Care (SBU 2003; Läkemedelsverket 2007). There is an indication for treatment with antiresorptive agents if the patient has previously suffered from a low energy fracture or if the *T*-score is $\leq$ –1.0 SD according to the expert group of the Swedish Medical Drugs Agency.

Other drugs that have been associated with impaired bone health include some antiepileptic drugs, heparin, aromatase inhibitors used in the treatment of breast cancer, GnRH treatment of prostate cancer. Also some modern antidiabetic drugs such as rosiglitazone have been associated with increased fracture risk. (Hawkins and Evans 2005; Chien and Goss 2006; Smith 2006; Petty, O'Brien et al. 2007; Richter, Bandeira-Echtler et al. 2007).

1.8.2 Osteoporosis secondary to other diseases

Many endocrine disorders affect the bone metabolism. Most common of these are primary hyperparathyroidism and thyrotoxicosis. Also Cushing's syndrome is associated with osteoporosis, mediated through the adverse effect of glucocorticoids. Diabetes mellitus which is non-insulin dependent has been associated in studies with both osteoporosis and higher bone mass compared with those not having diabetes. Insulin dependent diabetes is associated in most studies with reduced bone mass but not necessarily with increased fracture rate (Kelsey, Browner et al. 1992; Nicodemus and Folsom 2001).

Many diseases are associated with osteoporosis and increased fracture risk. These diseases include disorders with chronic malabsorption, chronic obstructive lung disease, renal failure and neoplasia affecting the skeleton, such as myelomatosis.

2 AIMS

2.1 GENERAL AIMS

The general aim of the PRIMOS project was to evaluate the prevalence of osteoporosis and the risk factors for osteoporosis and osteoporotic fractures among free-living elderly women in a primary care setting. The PRIMOS project has two parts, the cross-sectional study where the epidemiology and risk factors for osteoporosis are studied, and a randomised controlled clinical trial where the effect of low-dose estradiol on bone mineral density is studied.

2.2 SPECIFIC AIMS

2.2.1 Study I

The aim of study I was to investigate the relationship between calcaneal and axial bone mineral density in an elderly female population and to study the influence of different reference populations on the results of osteoporosis diagnosis.

2.2.2 Study II

The aim of study II was to determine whether there is an association between bone mineral density, as assessed by dual X-ray absorptiometry (DXA), and the nutritional status indicated by the Mini Nutritional Assessment method in a free-living elderly female population.

2.2.3 Study III

The aim of study III was to investigate the relationship between osteoporosis and calcium-regulating hormones and the growth factor IGF-I and its modulating binding protein IGFBP-1.

2.2.4 Study IV

The aim of study IV was to evaluate the effect on bone mineral density of transvaginal low-dose estradiol during a follow-up of two years.

3 MATERIAL AND METHODS

3.1 THE STUDY POPULATION

The women participating in the studies of this thesis all lived in the southern part of Stockholm, Sweden. The primary health care centre of Bagarmossen is located in the study area. The studies are parts of the PRIMOS (Primary Health Care and Osteoporosis) project and include cross-sectional studies of elderly women and a randomised clinical trial to evaluate the effect on bone mineral density of ultra-low doses of estradiol.

Figure 1 shows a flow-chart of the whole PRIMOS project.

A total of about 940 women born between 1920 and 1930 lived in the study area. Initially 300 women were randomly selected from this population and sent letters with information about the study and an invitation to participate. A second invitation was sent to all of the remaining 284 women born between 1926 and 1930 to increase the number of participants. A total of 351, or 60% of the 584 invited women participated in the study. To further increase the number of participants in the clinical trial a special invitation was sent out to the remaining 353 women in the population to participate on condition that they were eligible to the clinical trial. This resulted in an addition of 42 women who participated in both study I and study IV. All the women were mobile and able to come to the primary health care centre and the hospital for the DXA measurements. This excluded most women living in closed care such as old people's homes.

A total of 393 women participated in the PRIMOS project. Of these 388 were measured with DXA.

The first woman was recruited to the PRIMOS project in March 1999 and the last in February 2001.

The population in Study I consists of the 393 women who participated in the PRIMOS project. Five subjects not measured with DXA were omitted from the statistical analysis.

The population in Study II was identical to the population of Study I with the exception that the 42 women recruited on condition of being eligible for the clinical trial were excluded from this study. The population in Study II consists of 351 women.

The study population in Study III is identical to the study population in study II with the exception that one woman was excluded from the statistical analysis due to an extremely high level of parathyroid hormone (775.2 pg/ml caused by kidney failure).

The study population in the RCT (Study IV) consists of 115 women. Of these women, 73 women are also participants in the population-based cross-sectional study. In order to get enough participants for the RCT, the last 42 women were recruited from the same population on condition that they were eligible for the clinical trial, i.e. only women who did not meet any of the exclusion criteria of RCT were invited to come to a visit to the study centre. **Figure 2** shows the flow of participants through the RCT.

The first woman was randomised in June 1999 and the last in March 2001.

3.1.1 The analysis of non-participants

A drop-out analysis was performed to see whether the non-participants in studies II and III differed from the participants. A questionnaire was sent to all women who declined to participate. This questionnaire was answered by 46% of the non-participants. The women who participated were of a mean age (72.9 years) that was one year younger than that of the non-participants (73.9 years), a difference that was statistically significant.

Of both participants and non-participants, 7% already had the diagnosis of osteoporosis. A history of wrist fracture was more common among the participants (22.5%) than among the non-participants (12.0%) (p=0.047). In addition, more women among the non-participants (4.6%) than participants (1.4%) reported having suffered a hip fracture (p=0.053). The same percentage (35%) in both groups assessed their own health as being as good as that of others of approximately the same age, but more women among the participants (46.1%) than non-participants (19.4%) considered their health better than others of the same age.

Figure 1. Flowchart over the PRIMOS project

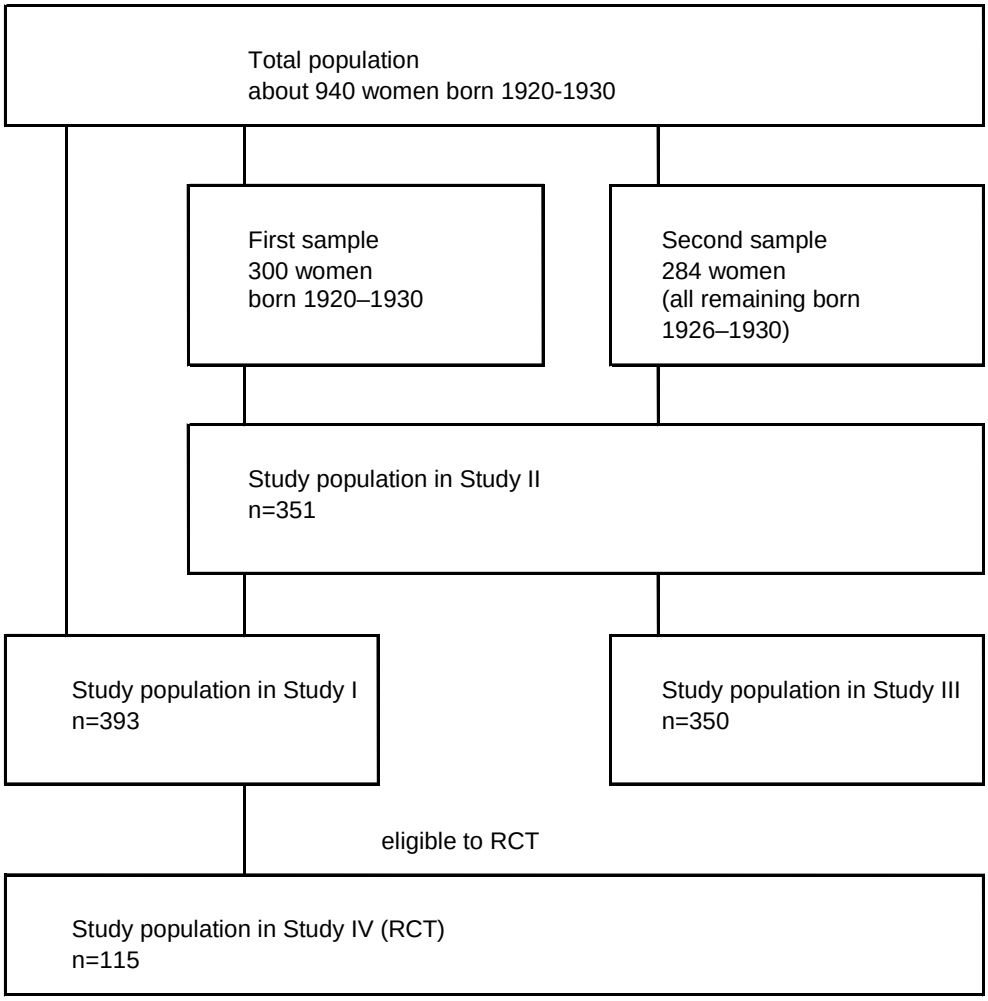
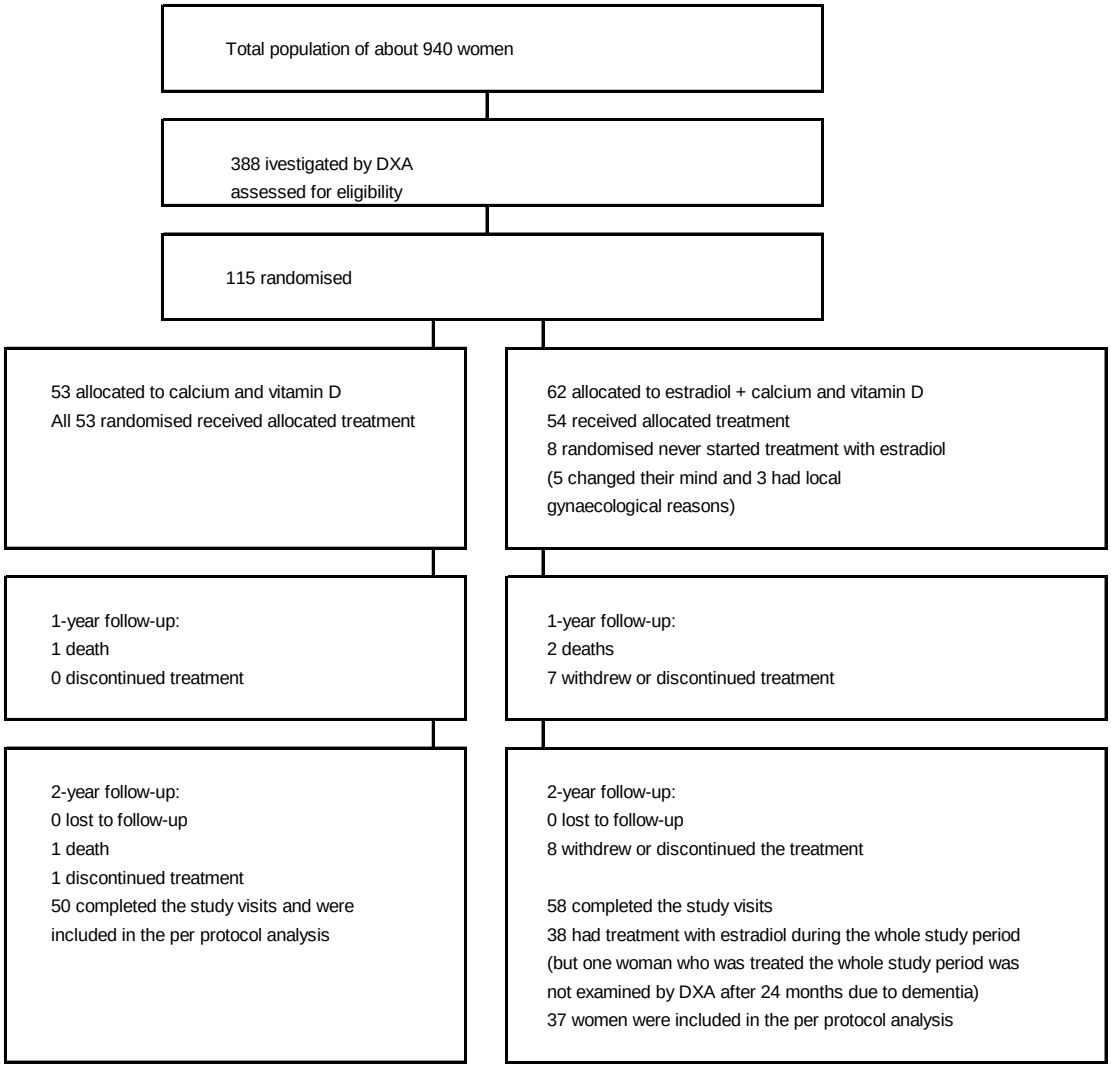


Figure 2. The flow of the participants through the RCT



Reasons for stopping treatment or withdrawing:

1. Before the end of the first year:

Control group: 1 died (liver cancer)

Estradiol group: 2 died (cardiac death), 1 breast cancer (discovered after 2 months of treatment, 1 endometrial hyperplasia (after 9 months of treatment), 1 pathological smear (discovered by the initial gynaecological examination), 2 vaginal discharge (after 1.5 and 3 months of treatment), 1 fear of estrogen (after 11 months of treatment), 1 local anatomical reason (after 6 months of treatment)

2. Before the end of the second year:

Control group: 1 died (thyroid cancer), 1 stroke

Estradiol group: 1 colon cancer (after 22 months of treatment), 1 breast cancer and stroke (after 14 months of treatment), 1 dementia (after 24 months of treatment), 2 vaginal discarge (after 15 and 20 months of treatment, 1 fear of estrogen (after 18 months of treatment), 1 withdrew consent (after 23 months of treatment), and 1 woman was never examined by DXA at the end of the study due to dementia.

All 115 randomised women were included in the intention-to-treat analysis.

87 out of 115 women were included in the per protocol analysis.

3.2 DXA AND DXL MEASUREMENTS

The bone mineral density of the lumbar spine and the hip were measured with Hologic QDR 4500 DXA equipment (Hologic Inc., Waltham, MD, USA).

In total 388 women had measurements of the spine, but in 6 women measurement of the hip was not possible due to bilateral hip-joint prostheses.

Lumbar spine measurements in L1–L4 (anteroposterior projection) were done in all subjects.

The BMD values for the central device were used to obtain *T*-scores and *Z*-scores with the NHANES III reference population for hip (Looker, Wahner et al. 1995). For lumbar spine the original Hologic reference population was used.

The bone mineral density of the calcaneus was measured in 388 subjects with DEXA-T (Stille AB, Stockholm, Sweden). The DEXA-T is an older version of the Calscan DXL device (Demetech AB, Solna, Sweden), which is commercially available today. The thickness of the heel is measured with laser beam reflection technology.

T-score values were obtained for the calcaneal device using the available reference population for the DEXA-T. *T*-score values with the Calscan reference population were obtained after converting the DEXA-T BMD values to corresponding Calscan DXL BMD values.

For details of DXA and DXL measurements, see Study I.

The WHO cut-off points were applied in order to classify subjects as normal (*T*-score $\geq$ –1 SD), osteopenic (*T*-score between –1 and –2.5) and osteoporotic (*T*-score $\leq$ –2.5).

Measurements in the RCT (Study IV) were repeated after 12 and 24 months.

3.3 EVALUATION OF RISK FACTORS FOR OSTEOPOROSIS

Risk factors for osteoporosis were evaluated during the study visit using a questionnaire that the women had answered at home prior to the visit, and additional questions during a face-to-face interview. The clinical status of the women was examined and their medication was evaluated.

3.4 MINI NUTRITIONAL ASSESSMENT (MNA)

We used the tool MNA to evaluate the nutritional status of the subjects. The MNA test contains 18 weighted questions divided into four categories:

1. Anthropometric measurements; four parts: measurements of the body mass index (BMI), mid-upper arm and calf circumferences and a question concerning weight loss during the past three months.
2. Global evaluation; six questions concerning accommodation type, medication, acute diseases, mobility, neuropsychological problems and pressure sores or skin ulcers.
3. Dietetic assessment; six questions concerning the number of whole meals eaten, food choice, intake of fluid per day and autonomy of feeding. To assess whether the food portions were sufficient the subjects were shown pictures with different portion sizes.

The choice of food concerned the selected consumption markers for protein intake such as dairy products, eggs and meat or fish. A protein score with three levels was based on this evaluation. Fluid intake was assessed by counting how many glasses containing 200 ml of fluid the subjects drank per day.

4. Self-assessment; two questions concerning sufficiency of food intake and self-perception of health. The subjects gave their personal view of having or not having nutritional problems and self-assessed their health in comparison with other people of the same age.

The maximum MNA score is 30 points. A score of < 17 points indicates malnutrition, 17–23.5 points indicates risk of malnutrition and a score of >24 points indicates adequate nutritional status.

One of the authors (H.S.) performed all the data collection during the participants' visit to the primary health care centre. The weight (kg), height (cm), mid-arm and calf circumference (cm) of the participating women were measured and they answered the questions orally during a face-to-face interview.

In a review of the studies where MNA has been used, Guigoz identified studies performed in over 30,000 elderly subjects in different settings (Guigoz 2006).

Of 13 studies addressing the sensitivity and specificity of the MNA instrument, only two had a sensitivity below 70% and the specificity range in the studies was 13–98% (Guigoz 2006). The lowest specificity of 13% (but a high sensitivity of 98%) was found in the study by Donini and colleagues, comparing MNA to nutritional assessment with anthropometry and serum proteins (Donini, de Felice et al. 2002).

The reliability of the MNA has been tested by Bleda and colleagues (Bleda, Bolibar et al. 2002) who found an intraclass correlation coefficient (ICC) of 0.89 testing the test-retest reliability and an internal consistency with Cronbach's Alpha of 0.83 and 0.74 at the second administration. The MNA was found to predict mortality in a follow-up study by Beck and colleagues of the Danish part of SENECA baseline survey (Beck, Ovesen et al. 1999). In a Swedish study, MNA predicted mortality in geriatric patients (Persson, Brismar et al. 2002). Saletti and colleagues found that the 3-year mortality among elderly receiving support was 50% for those identified as malnourished with the MNA instrument compared with a mortality of 28% for those identified as well-nourished (Saletti, Johansson et al. 2005).

The European Society for Clinical Nutrition and Metabolism (Espen) consider MNA to be one of the three best suited instruments to assess the nutritional status of the elderly (Kondrup, Allison et al. 2003).

3.4.1 Laboratory measurements

Fasting serum and urine samples were stored frozen at –70ºC until analysed. The baseline and on-treatment measurements were analysed with same assays.

Parathyroid hormone, 25-hydroxy vitamin D. IGF-I, IGFBP-1, glucose, cholesterol, triglycerides and calcium-status were measured in serum in all participants.

Estradiol, SHBG, CTx, U-Dpd and other markers were followed in the RCT.

For details of laboratory methods, see Methods sections of Study III and Study IV.

3.4.2 The study design of the RCT (Study IV)

The design was an open-label, randomised, parallel-group study.

The inclusion criteria for the RCT were a *T*-score value showing osteopenia or osteoporosis in the femoral neck or in the lumbar spine.

The exclusion criteria were: suspicion of secondary osteoporosis, diabetes mellitus of type I or diabetes mellitus type II with late manifestations of clinical importance, uncontrolled hormonal disorder of the thyroid or parathyroid, disease with gastrointestinal malabsorption (lactose intolerance not included), chronic obstructive lung disease requiring the use of more than 800 µg of inhalation steroids or treatment with oral steroids during the last year, continuous treatment with antiepileptica or continuous treatment with oral steroids in the last 5 years, clinically manifest renal insufficiency, known malignant disease, previous estrogen-dependent malignancy, treatment with bone active substances (calcium and vitamin D therapy not included), treatment with any preparation of estrogen during the last year, psychiatric disease or other disturbance that makes it difficult to adhere to the study protocol, previous hysterectomy or a large prolapse. Current smokers were excluded.

The women were assigned through a blocked randomised procedure to treatment with either a vaginal ring releasing 17 β-estradiol (average dose 7.5 µg/day) and a daily tablet containing 500 mg of calcium and 400 IU of vitamin D3 or treatment with 500 mg of calcium and 400 IU of vitamin D3. The randomisation list was computer-generated and organised by a statistician who also prepared numbered sealed opaque envelopes that were consecutively opened after the subject was included. The randomisation procedure was blinded to the investigator. In the control group, no placebo ring was used, because a ring that does not release estrogen might cause ulceration in the atrophic vaginal mucosa.

The primary outcome was bone mineral density (BMD) of the hip (femoral neck and total hip) and lumbar spine, which was measured at baseline and then annually for two years. The women in the estradiol-treated arm had endometrial thickness assessed with ultrasound at baseline and after one and two years. All women were offered a mammogram. The adherence to intake of calcium and vitamin D was assessed by counting pills at every visit. If assistance to change the vaginal ring was required, it was provided by one of the investigators (H.S.).

3.4.3 Treatment in the RCT

The vaginal ring Estring® (Pfizer) contains 2 mg micronised 17 β-estradiol in a core of a soft silicone ring. The outer diameter of the ring is 55 mm and the inner diameter is 9 mm. The vaginal device releases approximately 7.5 µg per day of estradiol, resulting in a total dose of 0.7 mg estradiol released continuously during its lifetime of three months. During the first three days of treatment higher doses of estradiol are released but the steady-state levels during continuous treatment increase only marginally (Schmidt, Andersson et al. 1994).

Both groups were treated with a daily tablet Calcichew D3® (Nycomed) containing 500 mg calcium and 400 IU vitamin D3.

3.5 STATISTICS

All the statistical analyses in the PRIMOS project were performed with STATA statistical software versions 7–9 (Stata Corporation, College Station, TX, USA) and SAS release 9 (SAS Institute Inc, Cary, NC).

The value of acceptance for statistical significance was set at $p < 0.05$.

3.5.1 Study I

Linear regression was used to study the relationship between different sites of DXA measurement. The correlation between *T*-scores at different sites of measurement was studied with the Pearson correlation coefficient. Kappa coefficients were calculated to estimate the interrater agreement between the heel measurements and the axial sites. Receiver operator characteristic (ROC) curves were produced to evaluate the sensitivity and the specificity of calcaneal DXL measurements with axial DXA used as the reference. The short-term precision CV_{SD}% was calculated using the formula proposed by Gluer and colleagues (Gluer, Blake et al. 1995). The precision of the heel bone densitometer over a period of three weeks was studied with analysis of variance (ANOVA) with repeated measurements and the method of Greenhouse-Geisser correction of the degrees of freedom.

3.5.2 Study II

In the analysis of the MNA questionnaire the only subject identified as malnourished was grouped together with the subjects at risk of malnutrition.

Unconditional logistic regression (Kleinbaum and Mitchel 2002, 1994) was used to analyse the relationship between osteoporosis and the items in the MNA questionnaire.

The items in the MNA questionnaire omitted from the model were not able to significantly improve the model. The logistic regression model was tested for interactions and for collinearity. Likelihood ratio test was performed for model improvement with a p-value <0.05 considered as significant model improvement. Model fit was tested with Pearson's goodness-of-fit test. A p-value > 0.05 was judged as a good fit. The results are shown as odds ratios (OR) with 95% confidence intervals (CI).

The outcome variable

The outcome variable in the logistic regression model was a *T*-score of $\leq$ 2.5 SD at the femoral neck and/or total hip dichotomised into osteoporotic or not osteoporotic (reference).

Explanatory variables

The MNA score dichotomised with the median score of 27 used as the cut-off point (reference MNA score $\geq$ 27).

1. Age dichotomised to under 75 years (reference) or over 75 years.
2. Weight categorised into: 45–55 kg, 56–69 kg (reference) and 70–126 kg. Weight and not BMI was analysed in the logistic regression model because weight showed higher association with bone mineral density than BMI.
3. MAC (mid-arm circumference in cm) categorised into: 22–27 cm, 28–31 cm (reference) and 32–47 cm.
4. Medication, the dichotomous variable of having or not having (reference) more than three drugs per day.

3.5.3 Study III

The relationships between the bone mineral density and the biochemical parameters were evaluated by analysis of multiple linear regression, after logarithmic transformations of variables with a skewed distribution (IGF-I, IGFBP-1, PTH, 25(OH)D and glucose). Pearson correlation coefficients and, when appropriate, Spearman rank correlation coefficients were calculated.

Logistic regression (Kleinbaum and Mitchel 2002, 1994) was employed to analyse the relationship between the outcome variables and the risk factors and risk markers.

The outcome variable osteoporosis was dichotomised into osteoporotic (*T*-score $\leq$2.5 SD) and non-osteoporotic (reference) at the femoral neck and/or total hip and/or trochanter and/or lumbar spine.

In the logistic regression models age and weight were used as continuous variables. Weight, and not BMI, was employed in the logistic regression model, since weight exhibited a closer association with bone mineral density. The logistic regression model was controlled for potential effect modifiers and controlled for collinearity. For model improvement the likelihood ratio test was carried out and a *P*-value <0.05 considered as indicating significant improvement. The model fit was examined using Pearson's goodness-of-fit test, with a p-value >0.05 judged as a good fit. The results are expressed as odds ratios (OR) with 95% confidence intervals (CI).

3.5.4 Study IV

A calculation of power was performed prior to the start of the study. The least significant difference of interest in bone mineral density was estimated at 3%, taking into account the expected bone loss in untreated women, the precision of the DXA technique and the effect of two years' treatment on bone mineral density in studies where fracture reduction had previously been shown (Chapuy, Arlot et al. 1992; Lufkin, Wahner et al. 1992; Black, Thompson et al. 2000). With a chosen significance level of 5%, a power level of 0.80 and an estimated standard deviation of 5%, we calculated that at least 45 subjects were needed in each group. The calculation of the sample size was based on a two-sample t-test. Considering an estimated dropout rate around 10%, we decided to include at least 50 women in each group.

Descriptive statistics were performed with Student's t-test for normally distributed continuous variables, Wilcoxon rank sum test for variables with a skewed distribution and Chi-square tests for categorical variables.

The estradiol values that were undetectable (10 pmol/L) were set to 5 pmol/L in the data analyses.

We analysed the primary outcome using mixed linear models procedure (Little and Raghunathan 1999). The dependent variables were BMD measurements at baseline, and after one and two years of treatment. The fixed effects were treatment group, time and treatment group time interaction showing the treatment effect of estradiol. The variables with skewed distribution were analysed after logarithmic transformation in the statistical analysis. An unstructured shape of covariance was used and the mixed models were adjusted for age.

3.6 ETHICS

Ethical approval was obtained from the Local Ethics Committee at Karolinska University Hospital Huddinge. Informed consent was obtained from the participants prior to their enrolment. Approval was also obtained from the Radiation Protection Committee at Karolinska University Hospital Solna.

Approval was also obtained from the Swedish Medical Drugs Agency for the randomised clinical trial with estradiol.

4 RESULTS

In this section only the main results are summarised. For more detailed results, please see the original articles.

Descriptive characteristics of the Primos population (n=351) that was the result of the two first samples are shown in **Table 1**.

Table 1. Descriptive characteristics of the population (n=351)

	All	Osteoporosis	Normal BMD/osteopenia	
	n=351	n=129		
Risk factor	**%**	**%**	%	P-value*
heredity	9.5	11.3	9.0	0.5
previously investigated by DXA	7.7	9.3	6.7	0.4
history of fracture (at any age)	41.0	54.3	31.3	**<0.0001**
history of fracture after the age of 50 years	30.9	43.4	22.7	**0.0001**
history of radius fracture	22.5	31.8	17.1	**0.0016**
history of hip fracture	1.4	2.3	0.5	0.1
current smoking	15.4	21.7	11.9	**0.015**
former smokers	28.2	21.7	32.2	**0.037**
Pharmacotherapy				
loopdiuretics	8.8	3.9	11.9	**0.012**
thiazide	8.0	12.4	5.7	**0.029**
betablockers	19.7	13.2	24.2	**0.014**
estrogen (any preparation)	33.9	24.0	40.3	**0.0022**
estriol	18.2	15.5	19.9	0.3
calcium and vitamin D	7.4	10.9	5.2	**0.05**
bisphosphonates	2.3	3.1	1.4	0.3
Diseases				
diabetes	8.3	5.4	10.4	0.1
hypertension**	21.7	21.7	21.8	1.0
heart disease**	17.1	7.8	22.8	**0.0004**
breast cancer	8.3	6.2	10.0	0.2
asthma or chronic obstructive lung disease	10.3	10.9	9.5	0.7

	All	Osteoporosis	Normal BMD/osteopenia	
Parameter	**Mean (Sd)**	**Mean (Sd)**	**Mean (Sd)**	P-value*
age (years)	72.9 (2.4)	73.2 (2.5)	72.6 (2.3)	**0.026**
weight (kg)	69.8 (12.1)	63.9 (10.8)	73.2 (11.0)	**<0.0001**
height (cm)	161.8 (5.9)	160.5 (5.9)	162.7 (5.8)	**0.0009**
blood pressure, systolic (mmHg)	157.6 (21.1)	159.6 (20.1)	156.3 (20.5)	0.2
blood pressure, diastolic (mmHg)	82.4 (9.0)	82.9 (8.9)	81.9 (8.9)	0.3
body mass index (kg/cm^2)	27.1 (4.5)	25.2 (4.1)	28.2 (4.3)	**<0.0001**
age at menopause (years)	49.5 (4.8)	48.7 (5.0)	50.0 (4.5)	**0.013**
age at menarche (years)	13.9 (1.5)	14.0 (1.3)	13.8 (1.5)	0.2
BMD femoral neck (g/cm^2)	0.649 (0.102)	0.569 (0.067)	0.697 (0.088)	-
T-score femoral neck	-1.8 (0.9)	-2.6 (0.6)	-1.4 (0.8)	-
BMD total hip (g/cm^2)	0.802 (0.1263)	0.702 (0.098)	0.863 (0.101)	-
T-score total hip	-1.2 (1.7)	-2.1 (0.9)	-0.7 (0.9)	-
BMD Wards triangle	0.452 (0.120)	0.369 (0.076)	0.502 (0.114)	-
T-score Wards triangle	-2.3 (1.0)	-3.0 (0.6)	-1.9 (0.9)	-
BMD lumbar spine (g/cm^2)	0.873 (0.162)	0.735 (0.102)	0.957 (0.127)	-
T-score lumbar spine	-1.6 (1.5)	-2.8 (0.9)	-0.8 (1.2)	-
BMD left heel (g/cm^2)	0.335 (0.060)	0.296 (0.049)	0.359 (0.050)	-
T-score left heel	-2.4 (1.0)	-3.0 (0.8)	-2.0 (0.8)	-

* P-value between the women with osteoporosis and the women with normal or osteopenic BMD
Osteoporosis defined as aT-score $\leq$ -2.5 at femoral neck and/or total hip and/or trochanter and/or lumbar spine
** The percentage of subjects with heart disease and hypertension is slightly higher compared with the results in Table 2b Study II due to additional information from the electronic patient records

4.1 STUDY I

Five subjects in the population who had no measurements with DXA were omitted from the analysis. The 388 participants investigated by DXA and DXL had a mean age of 73.1 years (SD 2.8).

The BMD values in both the femoral neck and the calcaneus decreased significantly with age ($p<0.001$).

The influence of the different reference populations on T-scores

The mean BMD values and the mean *T*-score and the *Z*-score values with the different reference populations differed a great deal. The percentage of subjects with osteoporosis using the *T*-score cut-off point at –2.5 SD differed from only 7.3% at the trochanter (using NHANES III reference population) to 53.4% at the femoral neck (using the Hologic original reference population).

ROC analyses of *T*-scores for osteoporosis in the heel related to osteoporosis at other sites showed an AUC (area under curve) of 0.802 for the femoral neck (sensitivity 78.4% and specificity 64.6%). The largest AUC of 0.906 was found for the total hip *T*-scores (sensitivity 92.2% and specificity 61.9%).

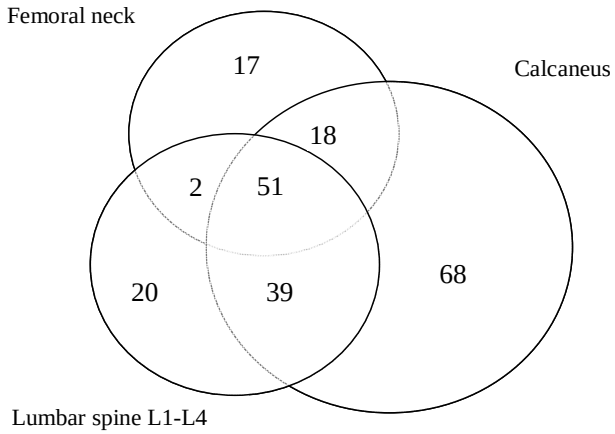


Figure 3. A Venn diagram showing the number of subjects with osteoporosis at the three sites measured and how the results overlap. The NHANES III reference population was used for femoral neck *T*-scores and the original Hologic reference population for the lumbar spine *T*-scores. The Swedish Calscan reference population was used for the heel *T*-scores. The total number of subjects with osteoporosis at any site was 215. Of these 215 subjects, 147 had osteoporosis in the femoral neck and/or lumbar spine. Some 74% or 108 of these were identified by the calcaneal measurement. At the same time, there were 68 out of 176 or 39% false positive calcaneal measurements when using the hip and/or the lumbar spine values as the reference.

The sensitivity of the DXL of the calcaneus in detecting axial osteoporosis varied between 13% and 92% depending on the DXA site or combination of sites and the choice of reference population.

Changing the reference population from Hologic to NHANES III influences the slope and the intercept in the regression analysis but the explanatory grade and the Pearson correlation coefficients show only minimal changes. The Pearson correlation coefficient between femoral neck *T*-scores (using NHANES III reference population) and *T*-scores at the left calcaneus was 0.604, the explanatory grade (R^2) 36%, between *T*-scores at total hip and left calcaneus 0.696 (R^2 48%). Similar correlations were found between femoral neck and lumbar spine (Pearson corr coefficient 0.619, R^2 38%).

Sensitivity and specificity

Figure 4a and **Figure 4b** show a graphic representation of the specificity and the sensitivity of different cut-off points for heel *T*-scores for detecting osteoporosis at the total hip and at the combination of the lumbar spine and the femoral neck. The optimal accuracy, the cut-off point in the heel *T*-scores where the curves for sensitivity and specificity crossed, was found at a *T*-score of –2.6 SD for detecting osteoporosis at the femoral neck. The same cut-off point of –2.6 in heel *T*-scores was found for the lumbar spine and for the combination of the lumbar spine and the total hip. For the total hip the cut-off point in the heel *T*-scores was between –2.8 and –2.9 SD. A heel *T*-score cut-off point of –2.0 gave a 90% sensitivity level for most central sites or combinations of sites. The only exception was the total hip which had a 90% sensitivity level cut-off point at the heel *T*-score of –2.5.

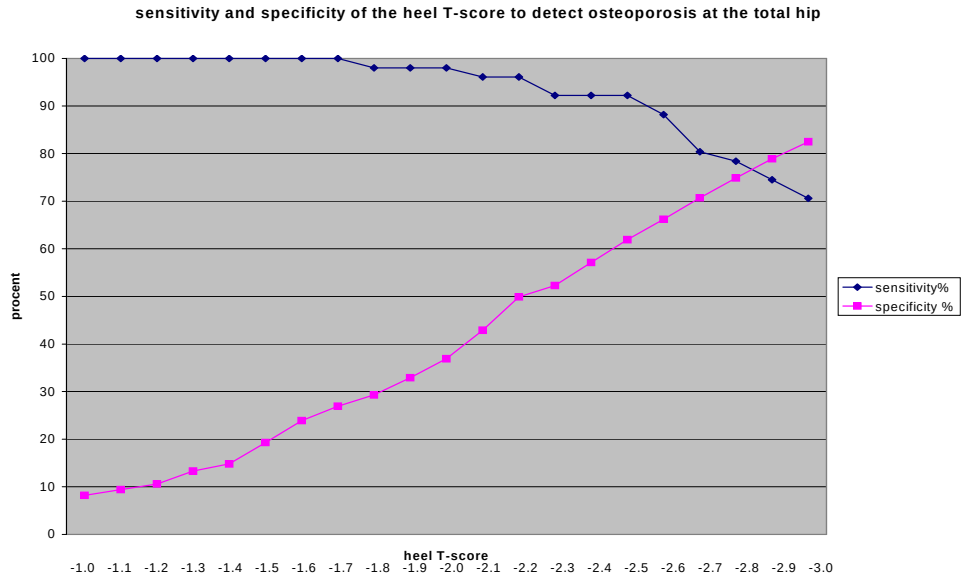


Figure 4a. The specificity and the sensitivity of the *T*-score at the left calcaneus for detecting osteoporosis in the total hip. The optimal cut-off point between specificity and sensitivity lies between heel *T*-score –2.8 and –2.9. The NHANES III reference population was used to calculate the *T*-scores at the femoral neck and the Calscan database was used to calculate the heel *T*-scores.

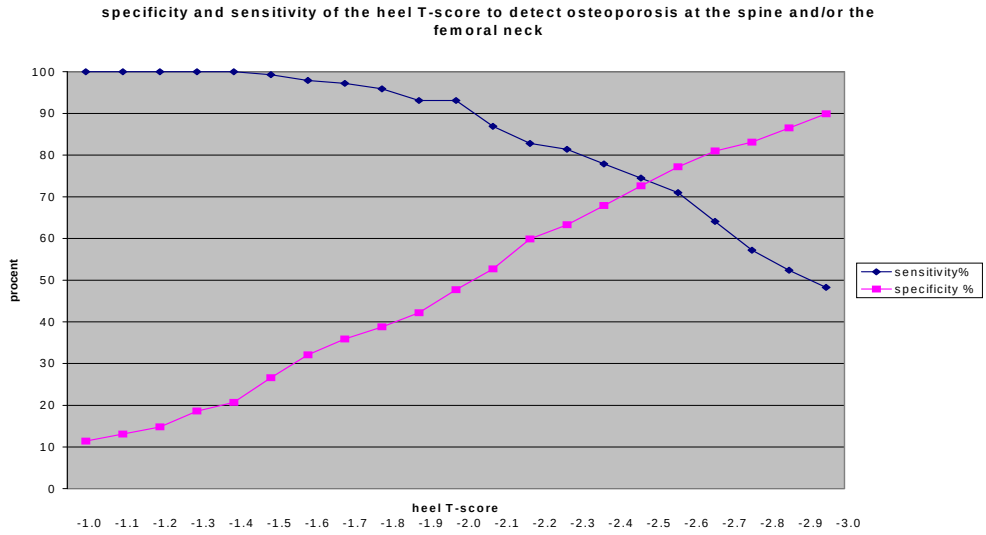


Figure 4b. The specificity and the sensitivity of the *T*-score at the left calcaneus for detecting osteoporosis in the lumbar spine and the femoral neck combined. The NHANES III reference population was used to calculate the *T*-scores at the femoral neck and the Calscan database was used to calculate the heel *T*-scores.

4.2 STUDY II

The participants in this study exhibited a high median MNA score of 27 (range 12.5–30). Only one subject could be considered malnourished; 7.4% (27) were at risk of malnutrition; and the nutritional status of the remaining 92.4% (323) was adequate according to the MNA.

The characteristics of the 351 participants are documented in **Table 2**. Forty per cent of the women with MNA scores $\leq$ 23.5 had osteoporosis at the femoral neck compared with 21% of the women with higher MNA scores. At total hip, the difference was even greater, 32% of the women with MNA scores $\leq$ 23.5 had osteoporosis at the total hip compared with only 10.8% of those with higher MNA scores.

Table 2. Descriptive characteristics of the 351 women partcipating in Study II

	All women (n=351)		MNA≤23.5 (n=28)		MNA≥24 (n=323)	
Parameter	Mean	SD	Mean	SD	Mean	SD
age (years)	72.9	±2.4	74.1	±2.4	72.8	±2.3
weight (kg)	69.8	±12.1	65.1	±3.8	70.2	±11.9
height (cm)	161.8	±5.9	159.6	±5.5	162.0	±5.9
body mass index (kg/m^2)	27.1	±4.5	25.9	±5.5	27.2	±4.4
age at menopause (years)	49.5	±4.8	48.2	±4.9	49.6	±4.8
age at menarche (years)	13.8	±1.5	13.8	±1.4	13.9	±1.5
T-score femoral neck	-1.8	±0.9	-2.5	±1.1	-1.8	±0.9
Z-score femoral neck	0.3	±1.0	-0.1	±1.2	0.3	±1.0
T-score total hip	-1.2	±1.2	-1.9	±1.5	-1.2	±1.1
Z-score total hip	0.6	±1.1	0.0	±1.4	0.7	±1.1
T-score lumbar spine	-1.6	±1.5	-1.9	±1.4	-1.7	±1.5
Z-score lumbar spine	0.7	±1.5	0.5	±1.4	0.7	±1.5
T-score left heel	-2.4	±0.9	-3.0	±1.3	-2.3	±0.9
Z-score left heel	-0.1	±0.9	-0.6	±1.2	0	±0.8
		percentage		percentage		percentage
heredity		9.5		10.5		9.4
diagnosis of osteoporosis prior to the study		7.1		17.7		6.2
previously examined by DXA		7.7		10.7		7.4
history of fracture (at any age)		41.0		42.7		40.9
history of fracture after the age of 50		30.9		42.3		30.0
history of wrist fracture		22.5		14.3		23.2
history of hip fracture		1.4		3.6		1.2
current smoker		15.4		14.3		15.5
former smokers		28.2		28.6		28.2
consumption of cheese: never		2.0		11.1		1.2
1-2 times a week		11.7		14.8		11.5
3-5 times a week		28.6		18.5		29.4
6-7 times a week		57.7		55.6		57.9
consumption of alcohol: never		34.6		59.3		32.5
1-2 times a week		58.9		37.0		60.7
3-5 times a week		5.4		3.7		5.6
6-7 times a week		1.1		0		1.2
consumption of milk, yoghurt (dl) mean (dl)		3.4		2.9		3.5

The proportion of our subjects not taking any medication at all on a regular basis was 16.2%.

The MNA questionnaire

The results of the MNA questionnaire showed that among the women at risk of malnutrition, more used drugs; acute illness, weight loss and/or psychological stress during the preceding three months were more common; few ate more than two complete meals each day; and 42.8% demonstrated a moderately reduced appetite.

The women appeared to be able to assess their own health status relatively well. In the group at risk of malnutrition 53.6% estimated their own health to be poorer than that of others of approximately the same age; while the corresponding figure for the well-nourished was only 6.4%. Among the women who evaluated their health as being better than that of others of the same age, 46.1% were well nourished while only 10.7% of those at risk of malnutrition made such a self-assessment.

Anthopometry

All of the anthropometric measurements i.e., BMI, weight and mid-arm and calf circumferences other than height exhibited highly significant correlations with the

BMD values. The Spearman correlation coefficient at all sites of measurement indicated a better correlation between BMD and weight than between BMD and BMI. The weight of the women correlated negatively with their age (Spearman correlation coefficient of –0.11, p=0.038); and their BMI values showed no significant correlation with their MNA scores (Spearman correlation coefficient of 0.05, p=0.374).

Logistic regression

In the final logistic regression model the outcome was dichotomised to osteoporosis or not osteoporosis (reference) at femoral neck and/or total hip.

Women weighing less than 56 kg exhibited a 2.5-fold elevation in risk of having osteoporosis compared to those with weight between 56–69 kg; and a body weight of more than 70 kg was seen to exert a protective effect. However, when the circumference of the mid-arm was also factored into the model, this influence of low body weight was no longer significant. Instead, a mid-arm circumference of less than 28 cm was the strongest risk factor in the case of the full model, being associated with a risk of osteoporosis which was three times higher than the risk of those in the reference category (i.e., with a mid arm circumference of 28–31 cm).

In the main effect model women with an MNA score of <27 demonstrated a twofold higher risk of osteoporosis compared to those with a score above this median. In addition, women taking more than three drugs daily demonstrated a twofold elevated risk in comparison to those using fewer drugs.

4.3 STUDY III

The women in the study had a mean value of 115 µg/L for IGF-I, 29 µg/L for IGFBP-1, 91 nmol/L for 25-OH vitamin D and 53 pg/ml for PTH.

Regression analysis and correlations

Table 3 shows the results of the regression analysis and correlation coefficients between the BMD values and selected parameters. We found an inverse relationship between the systolic blood pressure and the BMD at femoral neck, Ward's triangle and trochanter. The pulse pressure showed similar results while there was no significant correlation between the diastolic blood pressure and the BMD at any site of measurement. The glucose values of the women were significantly correlated to the BMD values of the total hip, lumbar spine and heel. We could not find any significant correlations in our study between the 25(OH)D values and the BMD. We could not find any seasonal variation in the 25(OH) D that was statistically significant. The regression analysis showed a significant inverse relationship between logarithmic values of PTH and the BMD values of the femoral neck after adjustment for age and weight (p=0.032).

Table 3. Analysis of regression and correlations between bone mineral density and age, weight and various factors
The BMD values are dependent in the analysis

	BMD site											
	Femoral neck				Total hip				Wards triangle			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*		Unadjusted		Adjusted*	
Parameter	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Age (years)	-0.0064	**0.006**	NA	NA	-0.0082	**0.004**	NA	NA	-0.0069	**0.011**	NA	NA
BMI (kg/m2)	0.0068	**<0.0001**	NA	NA	0.0115	**<0.0001**	NA	NA	0.0055	**<0.0001**	NA	NA
Weight (kg)	0.0031	**<0.0001**	NA	NA	0.0049	**<0.0001**	NA	NA	0.0025	**<0.0001**	NA	NA
Systolic blood pressure (mm Hg)	-0.0008	**0.003**	-0.0008	**0.002**	-0.0006	0.1	-0.0006	**0.028**	-0.0008	**0.010**	-0.0008	**0.010**
Diastolic blood pressure (mm Hg)	-0.0008	0.2	-0.0013	**0.027**	-0.0004	0.6	-0.0011	0.10	-0.0012	0.1	-0.0015	**0.031**
Pulse pressure (mmHg)	-0.0010	**0.003**	-0.0008	**0.006**	-0.0009	**0.033**	-0.0007	0.057	-0.0009	**0.018**	-0.0008	**0.037**
Glucose (mmol/L)	0.0465	0.1	-0.0163	0.6	0.1047	**0.008**	0.0090	0.81	0.0259	0.5	-0.0251	0.5
25-OH vitamin D	-0.0124	0.5	0.0171	0.3	-0.0296	0.1	0.01258	0.49	-0.0070	0.7	0.0147	0.4
PTH pg/ml	-0.0186	0.2	-0.0293	**0.032**	-0.0120	0.5	-0.0298	0.064	-0.0250	0.1	-0.0321	0.1

	Trochanter				Lumbar spine L1-L4				Heel			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*		Unadjusted		Adjusted*	
Parameter	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Age (years)	-0.0067	**0.008**	NA	NA	-0.0044	0.2	NA	NA	-0.0050	**<0.0001**	NA	NA
BMI (kg/m2)	0.0090	**<0.0001**	NA	NA	0.0137	**<0.0001**	NA	NA	0.0051	**<0.0001**	NA	NA
Weight (kg)	0.0038	**<0.0001**	NA	NA	0.0056	**<0.0001**	NA	NA	0.0024	**<0.0001**	NA	NA
Systolic blood pressure (mm Hg)	-0.0006	**0.042**	-0.0006	**0.024**	-0.0005	0.2	-0.0006	0.11	-0.0001	0.6	-0.0001	0.6
Diastolic blood pressure (mm Hg)	-0.0000	1.0	-0.0006	0.3	-0.0006	0.6	-0.0015	0.10	-0.0001	0.7	-0.0005	0.1
Pulse pressure (mmHg)	-0.0009	**0.011**	-0.0008	**0.020**	-0.0007	0.2	-0.0006	0.25	-0.0001	0.7	-0.00003	0.9
Glucose (mmol/L)	0.0586	0.1	-0.0173	0.6	0.1049	**0.037**	-0.0112	0.82	0.0595	**0.001**	0.0122	0.5
25-OH vitamin D	-0.0145	0.4	0.0188	0.3	-0.0381	0.1	0.0133	0.58	-0.0197	**0.035**	0.0019	0.8
PTH pg/ml	-0.0078	0.6	-0.0212	0.2	0.0313	0.2	0.0068	0.75	0.0061	0.5	-0.0019	0.8

In the analysis of regression, the parameters not normally distributed (glucose, 25-OH vitamin D and PTH) were analysed after log transformation
The analysis of regression of lumbar spine and heel was performed on 345 subjects, the analysis of hip on 339 subjects
r=slope
* age- and weight-adjusted
NA = non-applicable

Table 4 shows the results of the analysis of regression and correlation coefficients between metabolic risk factors, BMD and IGF-I and IGFBP-1. The IGFBP-1 values showed a highly significant inverse relationship to the BMD values at all sites of measurement, with Pearson correlation coefficients about 0.3. Figure 1 shows the regression fit between the BMD values of femoral neck and the logarithmic values of IGFBP-1. The IGF-I values showed a significant positive relationship to the BMD values at all sites except the lumbar spine. The glucose values were, as expected, significantly related to the IGF-I and IGFBP-1 values but we could not find any significant correlation between the 25(OH)D or the PTH values with either the IGF-I or the IGFBP-1 values. We also determined the ratio between IGF-I and IGFBP-1. The median ratio was 3.9 (range 0.7–37.4). This ratio correlated significantly with all sites of BMD measurement (p-values <0.001).

Table 4. Analysis of regression between cardiovascular risk factors, BMD and IGF-I and IGFBP-1
The values of IGF-I and IGFBP-1 dependent with the exception of the analysis of BMD where the BMD values are dependent

	IGF-I				IGFBP-1			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	r	P-value	r	P-value	r	P-value	r	P-value
Age (years)	-0.009	0.2	NA	NA	0.009	0.5	NA	NA
BMI (kg/m^2)	-0.001	0.8	NA	NA	-0.051	**<0.0001**	NA	NA
Weight (kg)	0.002	0.2	NA	NA	-0.020	**<0.0001**	NA	NA
Height (cm)	0.007	**0.011**	NA	NA	-0.003	0.6	NA	NA
Systolic blood pressure (mmHg)	-0.001	0.3	-0.001	0.4	-0.001	0.4	-0.001	0.4
Diastolic blood pressure (mmHg)	-0.001	0.5	-0.001	0.5	-0.009	**0.010**	-0.006	0.1
Glucose (mmol/L)	0.334	**0.001**	0.331	**0.001**	-0.409	**0.020**	0.032	0.9
BMD (g/cm^2)								
neck	0.057	**0.001**	0.045	**0.008**	-0.056	**<0.0001**	-0.033	**0.001**
total hip	0.071	**0.001**	0.052	**0.009**	-0.080	**<0.0001**	-0.044	**<0.0001**
trochanter	0.047	**0.017**	0.032	0.1	-0.066	**<0.0001**	-0.039	**<0.0001**
Wards triangle	0.084	**0.005**	0.047	**0.022**	-0.052	**<0.0001**	-0.036	**0.005**
lumbar spine L1–L4	0.054	0.1	0.038	0.2	-0.069	**<0.0001**	-0.023	0.2
heel	0.027	**0.010**	0.017	0.1	-0.035	**<0.0001**	-0.016	**0.004**
25-OH vitamin D (nmol/L)	0.053	0.3	0.072	0.2	0.039	0.7	-0.159	0.1
PTH (pg/ml)	-0.053	0.2	-0.053	0.2	0.002	1.0	0.096	0.2

In the analysis of regression, the parameters not normally distributed (glucose, 25-OH vitamin D and PTH) were analysed after log transformation
r=slope
* age- and weight-adjusted
NA = non-applicable

The Spearman correlation coefficient (0.246, p<0.001) was highly significant between the use of loop diuretics and the values of parathyroid hormone. About 20.0% of the subjects treated with loop diuretics had levels of parathyroid hormone above 65 pg/ml, compared with only 4.4% of the subjects with hormone levels below this cut-off point. Logistic regression showed that subjects using loop diuretics had a 4.4-fold elevated age- and weight-adjusted risk (p-value <0.001) of having levels of parathyroid hormone above 65 pg/ml compared to subjects with levels below 65 pg/ml. The logarithmic values of 25(OH)D and body mass index showed a significant inverse relationship (r= –0.024, p<0.001, the Pearson correlation coefficient –0.31). The values of parathyroid hormone and 25(OH)D also showed a strong inverse relationship (r= –0.220, p<0.001, Pearson correlation coefficient –0.198).

Additional analysis not previously presented in Study III
The percentage of women with a heart disease was significantly greater among those women who did not have osteoporosis. Only 8% of the women with osteoporosis had a heart disease, compared with nearly 23% of the women with normal BMD or osteopenia. We could not find any significant associations between the levels of IGF-I and IGFBP-1 and the presence of a heart disease in this study.

A logistic age- and weight-adjusted regression model was built where IGF-I and IGFBP-1 were dichotomised at the median level (the lower level reference). The outcome variable osteoporosis was dichotomised into osteoporotic (*T*-score $\leq$2.5 SD) and non-osteoporotic (reference) at the femoral neck and/or total hip and/or trochanter and/or lumbar spine.

The odds ratio for IGF-I levels above the median (OR 0.77 CI=0.47–1.26) was not statistically significantly protective. Those with IGFBP-1 levels above the median had a statistically significantly increased risk of having osteoporosis (OR 1.7 CI=1.03–2.83) compared with the women with IGFBP-1 levels below the median.

4.4 STUDY IV

A total of 115 women participated in the randomised clinical trial. Their mean age was 73.3 years. Osteoporosis in the femoral neck was found in 23.7% of the participants. There were no significant differences between the groups at baseline with the exception of urine deoxypyridinoline (p=0.021) and estradiol (p=0.028). Slightly more women, 23 vs 15, had hypertension in the control group.

The effect of estradiol on BMD

Table 5. Effect of estradiol on bone mineral density (g/cm^2), intention to treat analysis (n=115)
P-values from the mixed linear model procedure, adjusted for age

	Baseline BMD (g/cm2)		Final value 1 year		Final value 2 years		Test Interaction Time and Treatment
BMD Site	**Mean (SE)**	**P-value***	**Mean (SE)**	**P-value****	**Mean (SE)**	**P-value*****	**P-value**
Femoral neck							
estradiol	0.625 (0.012)	0.1	0.625 (0.013)	0.2	0.628 (0.013)	0.2	0.6
control	0.648 (0.010)		0.643 (0.010)		0.641 (0.010)		
Total hip							
estradiol	0.774 (0.016)	0.1	0.777 (0.017)	0.2	0.779 (0.017)	0.1	**0.04**
control	0.804 (0.015)		0.798 (0.015)		0.798 (0.014)		
Trochanter							
estradiol	0.606 (0.014)	0.6	0.610 (0.015)	0.8	0.614 (0.015)	0.7	0.3
control	0.613 (0.013)		0.606 (0.013)		0.609 (0.012)		
Lumbar spine L1-L4							
estradiol	0.828 (0.020)	0.2	0.843 (0.021)	0.5	0.850 (0.022)	0.3	**0.011**
control	0.861 (0.023)		0.868 (0.022)		0.880 (0.022)		
Heel							
estradiol	0.333 (0.007)	0.7	0.336 (0.008)	0.9	0.334 (0.008)	0.9	0.7
control	0.334 (0.008)		0.329 (0.008)		0.334 (0.008)		

* P-value for the difference between the estradiol and the control group at baseline
** P-value for the difference between the estradiol and the control group after one year
*** P-value fot the difference between the estradiol and the control group after two years

The changes in BMD over two years of treatment in the intention to treat analysis are shown in **Table 5.** The changes in BMD between the two groups were significant at total hip and lumbar spine. The estradiol-treated women increased in their BMD at lumbar spine by 2.6%, femoral neck by 0.5% and total hip by 0.6% compared to their baseline values, while the control group slightly decreased their BMD values at the femoral neck by 1.1% and total hip by 0.7% when compared to their baseline values. The lumbar spine BMD of the non-user group increased by 2.2%.

The effect of estradiol on biochemical markers
The CTx levels decreased by 38% in the estradiol group and by 26 % in the control group in the analysis of the intention to treat data (p=0.016), but the difference between the groups was not significant in the per protocol analysis (p=0.06). The estradiol levels in the per protocol analysis were significantly affected by the estradiol treatment (p=0.027), with an increase during the first year of treatment by 16.7% (5.03 pmol/L), followed by a decline by 15% (5.25 pmol/L) during the second year. There were no other significant treatment effects of estradiol on biochemical markers in the per protocol analysis. Both the estradiol group and the control group had lower levels of BALP (p<0.001) and U-Dpd (p=0.04) and PTH (p<0.001) and higher levels of vitamin D (p=<0.001) at the end of the study.

Safety and adherence to the medication
The ultrasound measurements of the thickness of the endometrium showed no significant increase in the thickness of endometrium during the study period of two

years. Apart from one case of endometrial hyperplasia, no more cases of abnormal vaginal bleeding were observed in the estradiol-treated group. One case of minor vaginal bleeding occurred in the control group. No woman in the estradiol treated group complained about breast tenderness or irritability. There was no statistically significant difference in the amount of urinary tract infections (11 versus 10) between the estradiol group and the control group. Four women in the estradiol group stopped treatment because of vaginal discharge with suspected vaginitis when examined by the study gynecologist.

In general, the women showed good adherence to the intake of calcium and vitamin D supplementation, assessed by counting the pills. They missed on average 11% of the calcium and vitamin D doses (range 0–56%). Adherence to the estradiol treatment was poorer; only 61% of the women allocated to the estradiol group had treatment with the vaginal device during the whole study period of two years. One of the investigators (H.S.) helped fifteen of the women to change their rings, while one woman visited her district nurse. We found no significant differences in the outcome between the women who changed their rings themselves and those who required assistance.

DISCUSSION

4.5 MAIN FINDINGS

The main finding of study I is that the WHO cut-off point of –2.5 SD seems applicable also for the peripheral BMD measurement of the heel. It supplies the optimal cut-off point for sensitivity and specificity for the heel *T*-score to detect osteoporosis at most central sites or combinations of central sites with the exception of total hip. Another main finding is that the comparison between central and peripheral measurements gives very different results depending on the chosen reference population. An adequate reference population is of the utmost importance for the diagnosis of osteoporosis based on *T*-scores.

The main finding of study II is that elderly free-living Swedish women of this age category have a generally good nutritional status, as determined by the Mini Nutritional Assessment, but even a slight deterioration in their MNA scores is associated with an increased risk of having osteoporosis.

In study III we found significant correlations between both IGF-I and IGFBP-1 and bone mineral density and the ratio IGF-I/IGFBP-1 ($p=0.002$). Some 26.6% of the women had elevated levels of parathyroid hormone ($\geq$65 pg/ml) that could indicate secondary hyperparathyroidism but the values were not higher in the women who had osteoporosis. Surprisingly, the values of 25(OH)D were slightly higher in the group of women with osteoporosis even after controlling for the possible intake of calcium and vitamin D supplementation. None of the women in our study had 25(OH)D levels below 25 nmol/L and only two subjects had levels below 30 nmol/L. We found no correlation either between the parathyroid hormone levels and the BMD or the levels of 25(OH)D and the BMD, but after adjustment for age and weight, regression analysis showed a positive association between the values of BMD at the hip and the logarithmic values of parathyroid hormone. Using loop diuretics was the most important factor contributing to secondary hyperparathyroisism in our study. Every fifth women with elevated parathyroid hormone levels in our study had treatment with loop diuretics.

The main finding of study IV was that there was a small but significant change in total hip and lumbar spine bone mineral densities during two years of treatment with transvaginal administration of 7.5 µg/24 hours of estradiol.

4.6 COMPARISON OF BMD RESULTS WITH OTHER STUDIES

Our study shows that measuring BMD with DXL in the calcaneus results in correlations and AUCs similar to those that have been reported previously in other studies using heel bone DXA (Fordham, Chinn et al. 2000; Langton and Langton 2000; Williams and Daymond 2003). The study by Fordham et al (Fordham, Chinn et al. 2000), using DXA technique for the heel and Lunar DPX-L for central measurements, showed a slightly higher correlation between BMD of the heel and the femoral neck, with $r=0.67$, and a weaker correlation with lumbar spine, with $r=0.69$. Fordham and colleagues found an AUC of 0.84 for the heel related to osteoporosis at the lumbar spine or femoral neck compared with the AUC of 0.81 in our study. The central measurements in the study by Fordham were made by Lunar DPX-L, and the

calcaneal measurements by PIXI DXA system. Using the same type of bone densitometers, Langton et al (Langton and Langton 2000) found an AUC of 0.81 for heel *T*-scores to detect osteoporosis at either the lumbar spine or the femoral neck. Williams et al. (Williams and Daymond 2003), who also used PIXI for calcaneal measurements, found an AUC of 0.89 for the left heel *T*-scores related to osteoporosis at the total hip, compared with the AUC of 0.91 in our study.

4.7 DIFFERENCES BETWEEN DIFFERENT SITES OF MEASUREMENT

The standard deviation of the mean of the differences between measurements in the calcaneus and the femoral neck or the total hip (0.84 and 0.85 respectively) is less than the standard deviation of the mean of differences between the femoral neck and the lumbar spine (1.16). Many women in this age group have reactive changes in the spine due to spondylarthrosis, thereby giving “falsely” higher BMD values in the spine. They may also have aortic calcifications that interfere with the analysis of the spine. This explains to some extent why the lumbar spine *T*-scores for individual women in our study differed from the femoral neck values by –2.5 to +4.1. In individual women in our study the femoral neck *T*-scores could differ from the calcaneal *T*-scores by –3.4 to +2.4, and the difference could be even greater between the lumbar spine and the calcaneus.

4.8 THE INFLUENCE OF REFERENCE POPULATIONS ON *T*-SCORES

Even small differences in a young adult reference population give a noticeable difference in *T*-scores at the –2.5 level (Faulkner, von Stetten et al. 1999). An accurate reference population is essential for the WHO-based diagnosis. The *T*-score values for the hip measurements changed dramatically after the change in reference population from the original Hologic population to NHANES III. The proportion of subjects classified as osteoporotic at the femoral neck decreased from 53% to 23% with the new reference population. The heel *T*-scores were also greatly affected when we calculated the new *T*-scores based on the new Swedish calcaneal database (Kullenberg 2003). The total number of subjects classified as osteoporotic varied from 7.3% (trochanter with NHANES III as reference population) to 53.4% (femoral neck with the original Hologic reference population) between the sites and with different reference populations. There seems to be some discordance in age-related BMD losses at different skeletal sites, as also indicated by our study, but the influence of the reference population on young adult *T*-score values is substantial, as shown in this and other studies (Lofman, Larsson et al. 1997; Faulkner, von Stetten et al. 1999; Sweeney, Malabanan et al. 2002). The original Hologic reference population and the NHANES III reference population are both North American, while both reference populations for the calcaneal device are Swedish. The NHANES III BMD values are considered fairly representative of Swedish women, as reported by Löfman et al (Lofman, Larsson et al. 1997). In fact, the BMD values for the age group 70–79 years that were found by Löfman were exactly the same as found in the NHANES III study. We therefore considerer it wiser to use the NHANES III reference population than the original Hologic reference population, while other studies also found large discrepancies compared with the NHANES III (Chen, Maricic et al. 1998). The standard deviation of the young adult mean in the Calscan reference population shows the same percentage of the peak bone mass (12.8%) as the NHANES III database for

the femoral neck BMD. This means that the *T*-score of –2.5 in both reference populations represents a BMD that is 32% below the young adult mean. Even small differences of the percentage of the SD of the young adult mean lead to discrepancies at the –2.5 level, as shown by Faulkner and colleagues (Faulkner, von Stetten et al. 1999). The same percentage of the young adult mean may be one of the explanations for the high agreement of the heel *T*-scores with *T*-scores at central sites seen in this study. The first reference population of the DEXA-T device had a young adult mean with a SD of 0.08 representing 15.6% of the young adult mean. In this reference population a *T*-score of –2.5 represented a BMD 39% below the young adult mean. This and the improvement of the technique explain the large differences between the two versions of the heel bone densitometer.

4.9 MEASURING THE HEEL – FOR SCREENING OR FOR DIAGNOSIS

Why measure the heel? Is it one more risk factor that can help the clinician refer the right patients for axial measurement, or can we rely on it for diagnostic purposes? A peripheral measurement gives information about the future fracture risk at a central site of measurement, but is this information good enough? A measurement at the site where the fracture is expected to occur gives the best information about the future fracture risk (Kanis, Johnell et al. 2004). Another problem is to find one cut-off point for all age categories. Finding a cut-off point for screening purposes in order to select patients for referral for central measurement might be easier. According to the International Society for Clinical Densitometry, if central BMD testing is available, patients who have a peripheral BMD measurement below the 90% sensitivity level should be tested further with a central BMD measurement (Miller, Njeh et al. 2002). Trying to find the cut-off point for optimal accuracy is another strategy used in studies. Fordham and colleagues suggested a *T*-score cut-off point in DXA calcaneus values of –1.3 in a younger population aged 60 years for the highest possible specificity (67%) and sensitivity (83%) to detect osteoporosis at the lumbar spine or at the femoral neck. Choosing the WHO cut-off point of –2.5 in the heel among older women in our study gave optimal accuracy, with both a relatively high sensitivity of 74.5% and a specificity of 73% for diagnosing osteoporosis at the femoral neck or the lumbar spine. If the original cut-off point of –2.5 SD was kept, the sensitivity of the calcaneal measurement for detecting osteoporosis at other sites or combinations of sites varied from 73 to 92% (*T*-scores calculated with the NHANES III and the Calscan database). These results differ from those obtained by Kullenberg and Falch, who found a sensitivity of 80% combined with a specificity of 82% for osteoporosis at the lumbar spine or at the femoral neck (Kullenberg and Falch 2003). If we want to use the peripheral BMD measurement for selecting patients to test further with central measurements, a cut-off point giving a sensitivity of 90% should be chosen, which is a heel *T*-score of around –2.0 for most sites and combinations of sites except for the total hip.

According to the 1994 WHO definition of osteoporosis (WHO 1994), the diagnosis of osteoporosis is based on a measurement at the hip, spine or forearm. Several studies have shown that a decrease of 1 SD in bone mineral density at the calcaneus has better predictive ability regarding hip fracture than a measurement at the forearm (measuring BMC) (Cummings, Black et al. 1993; Marshall, Johnell et al. 1996; Cummings, Bates et al. 2002).

It should also be remembered that the hip site for the original WHO cut-off values for osteoporosis thresholds was based on the femoral neck, and use of the classification was mainly for epidemiological purposes and not for clinical practice. In this study we have also related the heel *T*-scores to other densitometric definitions of osteoporosis.

We should differentiate between diagnostic thresholds and therapeutic thresholds. A decision to start treatment can be based on a peripheral measurement when there are other strong risk factors such as previous fractures, and in some cases the decision can even be made without any measurement of bone mineral density (Kanis and Gluer 2000.)

4.10 THE NUTRITIONAL STATUS WITH MNA

The fact that the elderly women living autonomously and participating in this study had a median MNA score of 27 points indicates that their nutritional status was good. Previous studies involving other tests for nutritional screening have also shown that only 1–5% of free-living elderly Swedes are malnourished (Thorslund, Toss et al. 1990; Cederholm and Hellstrom 1992). Many nutritional factors, such as protein intake, calcium and vitamin D, are known to affect bone mineral density, which is highly significantly correlated to body weight and body mass index. Many patients with osteoporotic hip fractures have previously been found to be undernourished and suffering from protein-energy malnutrition (PEM) (Ponzer, Tidermark et al. 1999). Osteoporosis is also a predictor of mortality (Johnell, Kanis et al. 2003) and, likewise, the MNA has been found to predict mortality in geriatric patients both during short-term stays in the hospital (Van Nes, Herrmann et al. 2001) and during a long-term three-year follow-up (Persson, Brismar et al. 2002).

The median MNA score of 27 points observed here is similar to that reported by others for elderly non-institutionalised individuals who have aged successfully (Scheirlinckx, Vellas et al. 1999; Maaravi, Berry et al. 2000).

The nutritional status of institutionalised elderly persons is much poorer, as illustrated by the report of Saletti and co-workers (Saletti, Lindgren et al. 2000). They found that approximately one third of elderly individuals living in assisted accommodation were malnourished as assessed by the MNA. In addition, patients seen by the general practitioners appear to differ in this respect from the free-living elderly in the general population. In the study by Beck (Beck, Ovesen et al. 2001) 38% of the patients with a mean age of 75 years coming from a Danish general practice obtained an MNA score of 17–23.5 points, indicating them to be at risk of malnutrition.

4.11 ASSOCIATIONS BETWEEN MNA AND OSTEOPOROSIS

Using the WHO criteria, osteoporosis was detected in the femoral neck in 22% of these subjects, but only one suffered from malnutrition as assessed by the MNA instrument. Surprisingly, this investigation reveals that even a slight deterioration in nutritional status is associated with an enhanced risk of osteoporosis. Women whose MNA score was below the median score exhibited a twofold higher risk of having osteoporosis in the femoral neck and/or total hip than those with a score above the median.

To our knowledge, no previous studies have described the possible association between bone mineral density (measured by DXA) and the nutritional status assessed by the MNA. One earlier study concluded that there was only a weak correlation and

trend between the calcaneal ultrasound parameters of BUA and SOS or Stiffness, respectively, and the MNA score (Gerber, Krieg et al. 2003). In this study by Gerber and coworkers, all the studied subjects were institutionalised and they demonstrated a much lower median MNA score than did our participants. The calcaneal bone mineral density in our subjects (measured by DXL) exhibited similar, but weaker, associations with various items in the MNA questionnaire than did the corresponding values for the hip and spine.

Unexpectedly, in our investigation, a small mid-arm circumference was associated with a higher odds ratio for having osteoporosis than low body weight. In fact, this former parameter was associated with the highest odds ratio, i.e., a threefold elevated risk of osteoporosis in women with a mid-arm circumference of less than 28 cm, compared to women with a corresponding circumference in the middle range. The main effect logistic regression model also indicated that a very large mid-arm circumference may be associated with an increased risk of having osteoporosis, but this observation was not statistically significant.

Our subjects who were regularly taking more than three drugs each day exhibited a twofold elevation in risk of osteoporosis in comparison to those using fewer drugs. Perhaps polypharmacy is an indicator of a general decline in health status that also affects the skeleton. For instance, Valtola and co-workers found that subjects regularly taking more than three drugs each day suffered twice as many ankle fractures during the 5-year follow-up period (Valtola, Honkanen et al. 2002).

4.12 OSTEOPOROSIS AND IGF- I AND IGFBP-1 STATUS

IGF-I has anabolic properties and is required for maintenance of bone health (Thoren, Hilding et al. 1998; Attanasio, Howell et al. 2002). Our results are in concordance with previous findings of Boonen and colleagues (Boonen, Lesaffre et al. 1996). The values of IGFBP-1 in our study showed significant association with the BMD values even after adjustment for age and weight at all sites with the exception of the lumbar spine. These findings are in discordance with data from the Rancho Bernardo study where no such association remained after adjustment for age and BMI (Jassal, von Muhlen et al. 2005). The levels of IGF-I in our subjects (mean 115 μg/L) were exactly the same as the Swedish reference values for this age group (115 μg/L) in the study by Hilding and colleagues (Hilding, Hall et al. 1999) but are lower than in the study by Unden and colleagues for the age group 60–74 years (142 μg/L) (Unden, Elofsson et al. 2002). This corroborates that the women in our study are good representatives of free-living elderly Swedish women.

4.13 OSTEOPOROSIS AND CALCIUM-REGULATING HORMONES

In our study of an elderly female Swedish population we found that 26.6% of the women had elevated levels of parathyroid hormone ($\geq$65 pg/ml) that could indicate secondary hyperparathyroidism, but the values were not higher in the women who had osteoporosis. Surprisingly, the values of 25(OH)D were slightly higher in the group of women with osteoporosis even after controlling for the possible intake of calcium and vitamin D supplementation. None of the women in our study had 25(OH)D levels below 25 nmol/L and only two subjects had levels below 30 nmol/L. The optimal level of 25(OH)D for all aims is still controversial and the different methods to measure 25(OH)D often show different results due to lack of cross-

calibration (Lips 2001). Chapuy and colleagues have proposed an optimal level of 25(OH)D of 78 nmol/L, below this level serum PTH started to rise (Chapuy, Preziosi et al. 1997). Some 33.7% of the women in our study had levels of 25(OH)D below 78 nmol/L. Analysis of regression showed a strong inverse relationship between the values of the parathyroid hormone and the values of 25(OH)D. There was also an inverse relationship between the values of BMI and 25(OH)D that has been shown in other studies as well (Wortsman, Matsuoka et al. 2000; Rucker, Allan et al. 2002; Arunabh, Pollack et al. 2003). We found no correlation either between the parathyroid hormone levels and the BMD or the levels of 25(OH)D and the BMD, but after adjustment for age and weight, regression analysis showed a positive association between the values for BMD at the hip and the logarithmic values of parathyroid hormone. Using loop diuretics has also previously been shown to be an important factor contributing to secondary hyperparathyroidism (Stein, Flicker et al. 2001). Every fifth women with elevated parathyroid hormone levels in our study had treatment with loop diuretics. The possible mechanism behind this effect of loop diuretics is that they increase the urinary loss of calcium.

4.14 THE EFFECT OF ULTRA-LOW DOSES OF ESTRADIOL ON BONE

This finding is in concordance with the previous study by Naessen and colleagues who found a significant increase by 2.1% in the forearm bone mineral density in the estradiol-treated group (Naessen, Berglund et al. 1997). The participants in the study by Naessen were fewer (n=30) and younger (mean age 66.8 + 1.4 years in the estradiol group), and the duration of the study was only six months and the women were not supplemented with calcium and vitamin D. Our study is the first where the longer-term effects of the estradiol-releasing ring over two years are studied and the first time its effect on bone mineral density of hip, spine and heel has been studied. It may be that 7.5 µg is near the lowest dose to have a significant effect on BMD because we failed to show a significant effect on BMD at all sites. Also one has to consider that the BMD of the lumbar spine increased among both the estradiol-treated women and the controls. A major part of that increase may be artificial due to increasing degenerative calcifications of the lumbar spine with increasing age. We may have missed a possible early positive effect during the first months because we did not measure change of BMD until after one year of treatment. The effect of this small dose of estradiol may decrease after some months, perhaps due to decreased absorption through the vaginal epithelium. We also saw a declining trend in our estradiol levels in the estradiol-treated group between one and two years, a decline also found in our per protocol analysis of the data. It is interesting that using a double dose of 14 µg estradiol transdermally, Ettinger and colleagues were able to show both a significant effect on hip and lumbar spine BMD, and increased levels of estradiol (Ettinger, Ensrud et al. 2004). One reason why we did not find treatment effect at all BMD sites might be that our study was powered to detect a difference of 3% between the groups, but treatment effect with estradiol doses in this low range may only result in a small increase of BMD. Even these small increases in BMD may be of interest, especially because these ultralow-dose treatments seem to have very few of the side-effects of conventional estrogen regimens. Previous pharmacokinetic studies of the ring have shown a mean increase in serum estradiol levels of approximately 2.5–3.9 pmol/L after a treatment period of four months to one year (Johnston 1996). Our

estradiol values also increased by 16.7% (5.03 pmol/l, per protocol data) after the first year and then showed a decline by 15.0% at the end of the study. This decline in estradiol levels during longer treatment with the ring could be explained by estradiol-induced maturation of the vaginal epithelium with reduced absorption of estradiol (Heimer and Englund 1984; Schmidt, Andersson et al. 1994) or could be due to increased metabolism of estradiol.

A non-oral route of administration of estrogen is theoretically appealing because it permits the use of much lower doses and has fewer side effects. Estrogen taken orally is metabolised by the hepatic cytochrome system, the so-called first-pass effect. Previous studies have shown that oral but not transdermal administration of estrogen reduces the circulating IGF-I levels (Weissberger, Ho et al. 1991). In fact, many of the effects of estrogen are route-dependent, and it seems that the transdermal or transvaginal route of administration is preferable. In our study, we could not observe any effect of estradiol on the levels of IGF-I or IGFBP-1.

4.15 LIMITATIONS

The cross-sectional studies have several limitations that should be mentioned. Only women who were able to come to the primary health care centre and to visit the hospital where the measurements of bone mineral density were performed, i.e., only women who were relatively mobile, were included. Another limitation is the cross-sectional design of our study, which does not allow us to examine causal relations. On the other hand, one strength is that our study group can be assumed to be representative of non-institutionalised women of this age group, since 60% of those invited to participate agreed and, in addition, nearly half of the non-participants answered the “drop-out analysis” questionnaire.

The randomised clinical trial also had limitations. It was not feasible to design the study with a placebo control since a placebo ring might cause ulcerations. Due to the costs, only women randomised to the estradiol arm had gynecological examinations. The advanced age of the participants and their frailty influenced the women's ability to participate during the whole study period of two years. The size of the study did not allow us to use fractures as an end-point. The study was only powered to detect a difference of about 3% increase in BMD. In this age group, a smaller increase might also be of clinical importance.

4.16 CONCLUSIONS

We conclude that BMD measurement of the calcaneus with DXL is a method that correlates fairly well to measurements at axial sites at the group level. In individual patients, large deviations were observed between all the measured sites. The change in reference populations has a substantial influence on the results of a comparison between different DXA methods. The peripheral bone densitometer is portable and easy to use in primary health care and for heel bone measurements in elderly people no longer living at home, who are at great risk of fractures. In the future, it could facilitate the selection of patients for further investigation using hip measurements. In addition, when used together with other strong evidence for fracture risk (such as previous fractures), it may be of value in making decisions concerning treatment. Our study population included only elderly women. Further fracture end-point studies and

studies in younger females and in males are needed before making general recommendations.

Elderly women with MNA scores under the median of 27 points, despite having a nutritional status that may be only slightly suboptimal, exhibit an increased risk of osteoporosis. The anthropometric measurements, especially mid-arm circumference and weight, contained in the MNA questionnaire are strongly associated with osteoporosis, but this questionnaire is not sensitive enough to be useful for clinical detection of osteoporosis.

The anabolic growth factor IGF-I and its binding protein IGFBP-1 had more influence on the BMD values of the women than vitamin D status and PTH status. The IGFBP-1 values were significantly inversely correlated to the BMD at all sites of measurement, and the levels of IGF-I were significantly positively correlated to the BMD at all sites with the exception of the lumbar spine. The BMD values were not correlated to the levels of 25-OH vitamin D of the women. The use of loop diuretics was the strongest risk factor for secondary hyperparathyroidism in this study. The users of loop diuretics had a more than fourfold elevated risk of having values of parathyroid hormone above 65 pg/ml compared with those not using loop diuretics.

The randomised clinical trial shows a positive effect on the bone mineral densities of total hip and lumbar spine of treatment with 7.5 µg of transvaginal estradiol. During the study period all women decreased in their bone turnover markers and PTH-levels while 25-OH vitamin D increased, a probable effect of the calcium and vitamin D supplementation. The decrease in the bone resorption marker CTx was significantly higher for the estradiol-treated women.

Future research is needed to establish whether these low dose regimens with estradiol designed for the local treatment of urovaginal atrophy also may also have a positive effect on fracture reduction.

5 FUTURE PERSPECTIVES

5.1 FURTHER ANALYSIS OF DATA FROM THE PRESENT STUDIES

Data remain to be analysed from the cross-sectional study and the RCT on the balance of the subjects. We also want to analyse further the relationship between osteoporosis and the other medical conditions of the women.

5.2 LONGITUDINAL FOLLOW-UP STUDY OF THE PRIMOS POPULATION

A longitudinal follow-up study of the whole population started in May 2007. In this study we have focus on the relationships between osteoporosis and cardiovascular mortality and mortality. We also get a better estimate of the predictive value of some of the estimates in the cross-sectional study, such as the nutritional status of the subjects and the biochemical risk markers.

6 SUMMARY IN SWEDISH

Bakgrund
Osteoporos är en folksjukdom som drabbar främst äldre kvinnor. Äldre svenska kvinnor tillhör de mest frakturdrabbade i världen. Bedömning och uppföljning av osteoporos bör i första hand ligga hos primärvården. I PRIMOS-projektet (Primärvård och osteoporos) studeras i tvärsnittsstudien riskfaktorer for osteoporos och frakturer hos äldre hemmaboende kvinnor med speciell fokus på olika metoder att mäta bentäthet och kvinnornas näringsstatus. Den andra delen av PRIMOS-projektet är en randomiserad klinisk prövning där vi studerar effekt på bentäthet av lågdosöstrogen administrerad via en vaginalring med en uppföljningstid på 2 år.

Delstudie 1 är en jämförelse mellan perifer bentäthetsmätning av hälen och konventionell bentäthetsmätning av höft och ländrygg. Den perifera mättekniken gör det möjligt att utföra bentäthetsmätningar ute på vårdcentralerna närmare patienterna till en lägre kostnad. Denna mätmetod av bentäthet med DXL (Dual X-ray and Laser) i hälbenet har dock inte blivit tillräckligt vetenskapligt utvärderad.

Delstudie 2 är en studie om relationen mellan äldre hemmaboende kvinnors näringsstatus bedömt med hjälp av MNA (Mini Nutritional Assessment) och kvinnornas osteoporosgrad mätt med bentäthetsmätning med DXA (Dual X-ray Absorptiometry). Låg vikt är en riskfaktor för osteoporos och många näringsfaktorer som D-vitamin och kalcium har stor betydelse för upprätthållandet av benstommen. BMI (body mass index, kg/m2) under 19–20 innebär en stor osteoporosrisk medan kvinnor som väger över 70 kg sällan är drabbade. MNA formuläret är enkelt att använda, tar kort tid att utföra, kräver inga laboratorieprover och kräver ingen annan utrustning än en våg, ett längdmätningsinstrument och ett måttband. Allt detta gör det till ett idealiskt instrument för primärvården.

Delstudie 3 har fokus på dels hur de kalciumreglerande hormonerna parathormon (PTH) och vitamin D förhåller sig till kvinnornas bentäthetsvärden, dels hur nivåerna av insulinets tillväxtfaktor IGF-I och dess bindande och effektmodifierande bärarprotein IGFBP-1 förhåller sig till kvinnornas bentäthet. Sekundär hyperparathyroidism till följd av brist på vitamin D och bristande absorption av kalcium från tarmen är ett vanligt tillstånd hos äldre kvinnor och påskyndar ytterligare benförlusterna. IGF-I är en av faktorerna som medverkar vid benformation i skelettet. Nivåerna av IGF-I minskar med stigande ålder och är korrelerade till nivåerna av bentäthet. Nivåerna av IGFBP-1 däremot stiger med åldern och det finns få studier om hur dessa nivåer förhåller sig till bentäthet.

Delstudie 4 är en randomiserad klinisk prövning där effekt av lågdosöstrogen studeras. Det har kommit under de senare åren flera studier där man har kunnat bevisa effekt på benmassa med betydligt lägre doser östrogen än vad som använts tidigare. Samtidigt har resultaten från speciellt två stora randomiserade kliniska studier med kombinerat östrogen och gestagenbehandling i konventionell dos i tablettform visat att den sammanlagda nyttan hos dessa behandlingsalternativ kanske inte överstiger

riskerna. Samtidigt finnas det idag övertygande evidens för att de endogena östrogennivåerna hos både män och kvinnor har stor betydelse för bevarandet av benstommen även i mycket höga åldrar. Många äldre kvinnor behöver skydd i form av östrogenpreparat mot sköra slemhinnor i underlivet. Om den här typen av lokalbehandling, som inte alls är förknippad med den riskprofil som konventionell östrogenbehandling är, skulle samtidigt medverka till att bevara kvinnornas benstomme, skulle man här få systemisk nytta av lokalbehandlingen. Därför är det av intresse att studera hur dessa lokabehandlingsmedel påverkar benstommen.

Syfte

Huvudsyftet med delstudie 1 var att utvärdera hur väl perifer DXA-mätning av hälen korrelerar med mätning i höft och ländrygg hos äldre kvinnor. Syftet var även att jämföra effekten av olika referenspopulationer för resultaten.

Huvudsyftet med delstudie 2 var att studera näringsstatus hos äldre hemmaboende kvinnor med hjälp av Mini Nutritional Assessment (MNA) och att studera relationen mellan försämrad näringsstatus och osteoporos.

Huvudsyftet med delstudie 3 var att studera hur nivåerna av dels parathormon och vitamin D, dels IGF-I och IGFBP-1 förhåller sig till bentäthet.

Huvudsyftet med delstudie 4 var att ta reda på om ett östradiolpreparat avsett för lokalbehandling av sköra slemhinnor hade systemisk effekt på benstommen under en behandlingstid på två år.

Material och metoder

Studiepopulationen

Kvinnorna som deltog i tvärsnittsstudien bodde alla i upptagningsområdet för Bagarmossens vårdcentral i Södra Stockholm. Den totala populationen kvinnor boende i området födda från och med 1920 till och med 1930 bestod av cirka 940 stycken kvinnor. Urvalet av kvinnor gjordes i två etapper. Först gjordes ett slumpmässigt urval på 300 kvinnor födda från 1920 till och med 1930 som fick information och ett erbjudande att delta. Av dessa kvinnor valde 179 att delta. Ytterligare en inbjudan skickades senare till alla resterande kvinnor födda mellan 1926 och 1930 vilket resulterade i ytterligare 172 deltagare. Totalt deltog 351 kvinnor i den populationsbaserade tvärsnittsstudien i delstudie 2. Av dessa 351 uteslöts en deltagare från delstudie 3 p g a ett extremt högt värde på parathormon. Det enda inklusionskriterium som tillämpades för den populationsbaserade delen av tvärsnittsstudien var att kvinnorna kunde ta sig till besöket på vårdcentralen och till sjukhuset för bentäthetsmätning, vilket uteslöt främst kvinnor i andra boendeformer samt hemsjukvårdspatienter. Inga exklusionskriterier utöver kravet på mobilitet tillämpades. För att rekrytera tillräckligt många deltagare till den randomiserade kliniska prövningen som utgjorde projektets andra del erbjöds resterande kvinnor i populationen att bli undersökta under förutsättning att kvinnorna uppfyllde kriterierna för inklusion till behandlingsstudien. Ytterligare 42 kvinnor rekryterades på detta sätt. Dessa kvinnor deltar även i delstudie 1 där bentäthetsmetoder jämförs.

Den första deltagaren gick in i kartläggningsstudien i mars 1999 och den sista i februari 2001. Kvinnorna fick ett brev med information om studien och ett

erbjudande om ett kostnadsfritt läkarbesök på vårdcentralen. Två påminnelser skickades ut till de kvinnor som inte svarat. Bortfallsanalys i enkätform utfördes bland kvinnor som inte deltog i studien.

Bentäthetsmätningar
De centrala bentäthetsmätningarna av höft och ländrygg utfördes på röntgenavdelningen på Karolinska universitetssjukhuset Solna med Hologic QRD 4500 utrustning med professor Hans Ringertz som ansvarig. De perifera bentäthetsmätningarna av hälbenet utfördes på endokrinologmottagningen på samma sjukhus med DEXA-T hälbenmätare.

Mini Nutritional Assessment
MNA består av 18 frågor grupperade i fyra områden:
1. Kroppsmått: BMI, överarmsomfång, vadomfång samt en fråga om viktnedgång de sista tre månaderna.
2. Allmän utvärdering: sex frågor rörande bostadstyp, mediciner, akut sjukdom, rörlighet, neuropsykologiska problem samt förekomst av trycksår.
3. Bedömning av näringsinnehåll: sex frågor rörande antal hela måltider, val av proteinrik föda, intag av vätska samt självständighet i ätsituationen.
4. Självbedömning: två frågor rörande om näringsintaget upplevdes som tillräckligt och om självuppskattad hälsa.

MNA poäng <17 tyder på malnutrition, >17 och <24 risk för malnutrition och >24 poäng på gott näringsstatus.

Etikprövning
Projektet godkändes av den humanetiska kommittén vid Huddinge sjukhus samt av strålskyddskommittén vid Karolinska sjukhuset. Den randomiserade kliniska prövningen med Oestring godkändes av Läkemedelsverket.

Resultat
Delstudie 1 visar att den perifera mättekniken av bentäthet med DXL av hälbenet överensstämmer relativt bra med centrala bentäthetsmätningar i höft och ländrygg i gruppen som helhet. Hos enskilda individer kunde stora skillnader ses mellan T-scorevärdet i hälben och höft. Samma WHO cut-off punkt –2.5 SD i T-score kan användas vid hälmätning för de flesta centralt definierade osteoporosdiagnostiska mätställen eller kombinationer av dessa med undantag för främst total hip. Studien visar också att bentäthetsmätarens referenspopulation har stort inflytande på andelen klassade som bensköra.

Delstudie 2 visar att äldre svenska kvinnor i eget boende har god näringsstatus mätt med MNA. Endast en studiedeltagare blev klassificerad som undernärd och bara 7.4 % låg i riskzonen för undernäring. Studien visar också att kvinnor i gruppen med MNA resultat under medianen 27 poäng har en nära tvåfaldigt ökad risk för att vara bensköra.

Delstudie 3 visar att nivåerna av den anabola tillväxtfaktorn IGF-I korrelerar positivt med kvinnornas bentäthet medan nivåerna av dess inhiberande bärarprotein IGFBP-1 ökar med minskande bentäthet.

Dessa faktorer var starkare korrelerade till kvinnornas bentäthet än kvinnornas halter av parathormon och vitamin D. Delstudie 3 visar också att relativt få kvinnor

hade brist på vitamin D medan nivåerna av parathormon var förhöjda hos cirka en tredjedel. Den största ökningen av parathormonvärden visade sig bero på samtidig användning av loopdiuretika som ökar kalciumförlusterna via njurarna och orsakar därigenom ökning av parathormonsekretionen.

Delstudie 4 visar att estradiol i form av vaginalringen Oestring®, som frisätter 0,075 mg estradiol per dygn, hade en liten men positiv inverkan på bentätheten under en uppföljningstid av två år.

Slutsatser och betydelse

Bentäthetsmätningar av hälbenet korrelerar på gruppnivå relativt bra med centrala bentäthetsmätningar i höft och ländrygg men på individnivå kan man hitta stora skillnader. Samma WHO cut-off punkt i T-score verkar gälla för hälen som för de flesta centralt definierade bentäthetsbaserade diagnostiska mätställen eller kombinationer av dessa. Det saknas dock kunskap om det frakturprediktiva värdet av hälmätningar enligt denna nya DXL teknik som gör att tekniken bör ytterligare utvärderas innan några mer allmänna rekommendationer kan ges.

Fyndet att kvinnor med endast en lätt försämrad näringsstatus mätt med MNA har en nära tvåfaldigt ökad risk att vara bensköra, jämfört med kvinnor som har MNA poäng över medianen, ger oss ytterligare en anledning att oftare använda MNA vid bedömningar av näringsstatus ute i primärvården. Redan små avsteg från optimalt MNA-poäng verkar vara förknippade med en ordentligt ökad risk för att vara benskör.

För äldre svenska kvinnor i den här studien verkar nivåerna av tillväxtfaktorn IGF-I och dess bärarprotein IGFBP-1 ha starkare relation till bentäthet än kvinnornas nivåer av parathormon och vitamin D. Brist på vitamin D var inte den dominerande anledningen till sekundär hyperparathyroidism i den här studien utan vad som hade större betydelse var den utbredda behandlingen med loopdiuretika.

Många äldre kvinnor är i behov av lokal östrogenbehandling mot sköra slemhinnor. Om denna behandling även medför positiva effekten för benstommen kan den göra nytta i flera avseenden.

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