Risk factors for bone fragility and fracture in postmenopausal women

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Abstract

The aim of this thesis was to evaluate risk factors for bone fragility and fractures in postmenopausal women in a long-term perspective. The studied sample was homogeneous and consisted of 390 north European women from a population-based cohort and the study period spanned from the age of 48 to age 82. At the start of the study, general health and lifestyle parameters were noted and bone measurements of the distal forearm were done by single-photon absorptiometry. Fractures were registered through hospital archives and regional databases and mortality through national population records. We found that women with menopause before age 47 had a higher prevalence of osteoporosis at age 77 and a higher incidence of fragility fractures and mortality during the follow-up period up to age 82. When other risk factors noted at baseline were included in the analysis, menopause before age 47 remained an independent risk factor for mortality, but not for fracture. Only low BMD predicted fracture risk independently, indicating that the predictability by early menopause is mediated by other factors, chiefly low BMD. The women who were still premenopausal at baseline were followed through their natural menopause which was determined according to the WHO criteria. The effects of physical activity were studied in 112 women who were followed with repeated SPA measurements for 25 years and reported their moderate physical activity in questionnaires. The women with more than 30 minutes of daily physical activity had a significantly lower annual bone loss during the follow-up period, and a higher bone mineral content at study end (age 77). The results remained after adjustment for age at menopause and postmenopausal oestrogen levels, and there were no significant group differences in lifestyle, diseases or medication. Postmenopausal changes in bone mass and bone structure were evaluated in eight-year intervals in 81 women without diseases or medication interfering with bone metabolism. During the first eight years after menopause, the annual bone loss was 2%, and there after 1% until study end (on average 24 years post-menopause). The periosteal width increased by 1% during the first eight years, but not subsequently. Bone loss had a stronger association with serum oestradiol levels than did the changes in periosteal width.

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Fracture, Osteoporosis, Bone Fragility, Bone strength, Periosteal expansion, Menopause, Physical activity

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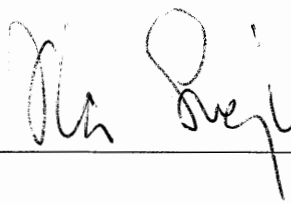
Signature  Date 28/2 2013

Risk factors for bone fragility and fracture in postmenopausal women

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To my family

“A mind once stretched by a new idea never regains its original dimensions.”

Oliver Wendell Holmes

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Early Menopause and Risk of Osteoporosis, Fracture and Mortality:
A 34-Year Prospective Observational Study in 390 Women
Svejme, O, Ahlborg, HG, Nilsson, J-Å and Karlsson, MK.
British Journal of Obstetrics and Gynaecology 2012 Jun; 119(7):810–816

Physical activity reduces bone loss in the distal forearm in post-menopausal women– A 25-year prospective study
Svejme O, Ahlborg HG and Karlsson MK.
Scandinavian Journal of Medicine and Science in Sport 2012 Jul 30 Epub ahead of print

Changes in Forearm Bone Mass and Bone Size after Menopause
– a Mean 24-Year Prospective Study
Svejme O, Ahlborg HG and Karlsson MK.
Journal of Musculoskeletal and Neuronal Interactions 2012; 12(4):192–198

Low BMD is an Independent Predictor of Fracture and Early Menopause of Mortality – A 34-Year Prospective Observational Study in 390 Women
Svejme, O, Ahlborg, HG, Nilsson, J-Å and Karlsson, MK.
Maturitas 2013 Jan 29 Epub ahead of print

Abstract

The aim of this thesis was to evaluate risk factors for bone fragility and fractures in postmenopausal women in a long-term perspective. The study period spanned from the age of 48 years to age 82 and is thus unique in its length. The studied sample was homogeneous and consisted of 390 north European women from a population-based cohort. At the start of the study, general health and lifestyle parameters were noted and bone measurements of the distal forearm were done by single-photon absorptiometry. The women who were still premenopausal were invited to enter a prospective perimenopausal study, and were followed through their natural menopausal transition by continuous endocrinological and bone measurements. Age at menopause could thus be determined exactly according to the criteria established by the World Health Organization (WHO). Bone measurements were done on average every second year during the first twenty years. At age 72, all women still remaining from the original cohort were invited to participate in a follow-up measurement, which was repeated at age 77. During the entire follow-up period, incident fractures were registered through repeated searches in hospital archives and databases, and mortality was registered through correspondence with the national population registers.

Our studies found that menopause before age 47 is a risk factor for osteoporosis, fragility fracture and mortality also in a long-term perspective. When the 390 women were dichotomised into categories of women with menopause before and after the age of 47, we found that the risk was significantly increased in the women with early menopause. When a list of other known risk factors were taken into account, menopause before age 47 remained an independent risk factor for mortality, but not for fracture. Only low BMD predicted fracture risk independently, indicating that the predictability of early menopause is mediated by other factors, chiefly low BMD. As regards the mortality risk, early menopause could either be the causal factor itself, or a result of complex underlying circumstances that lead to both early menopause and mortality. We therefore recommend women with early menopause to obtain advice on lifestyle and consider having their bone mass measured during the first decade after menopause.

The effects of physical activity were studied in 112 women who were followed through their menopause with repeated SPA measurements for 25 years on. The

women reported their level of everyday general moderate physical activity during the follow-up period in questionnaires, and were dichotomised according to a cut-off value of 30 minutes per day. The physically active women had a significantly lower rate of annual bone loss than the inactive women, and higher bone mineral content at study end (age 77). The results remained after adjustment for age at menopause and postmenopausal oestrogen levels, and there were no significant group differences in lifestyle, diseases or medication. These results suggest that physical activity could be a useful strategy for postmenopausal women to reduce the risk of bone fragility.

In 81 women who were followed through their menopause and participated in the repeated bone measurements, changes in bone mass and bone structure were evaluated in different time phases after menopause. During the first eight years after menopause, there was an oestrogen-related annual bone loss of 2%, followed by an age-dependent bone loss of 1% per year until study end (on average 24 years post-menopause). This was partially compensated by an annual increase in periosteal width of 1% during the first eight years, whereas during the rest of the study period, no periosteal expansion was found. These results could not be associated with fracture risk, but indicate that bone strength is partially preserved in the early postmenopausal period, by the increased bone width which counteracts the rapid bone loss.

Introduction

Osteoporosis and fragility fractures constitute a major health problem, in terms of both individual suffering and financial costs. Approximately, almost one quarter of all men and half of all women will develop osteoporosis and almost one half of all women will sustain an osteoporotic fracture [1, 79]. The last century has seen a steady increase in the fracture incidence, with an estimated number of 70,000 osteoporotic fractures annually in Sweden [2] Although the ever-growing proportion of elderly in the population is largely responsible for this exponential rise, this cannot totally explain the increase. Comparisons of bone specimens from two centuries indicate that women in general lose bone mass earlier in the modern world [88], perhaps owing to a more sedentary lifestyle, less parity, the introduction of smoking or a lower dietary intake of calcium. Despite the steady progress in the awareness and treatment of osteoporosis, hip fracture incidence in Scandinavia is still the highest in the world, although slightly decreasing the last decades [132]. The reason is unclear, but a number of risk factors need to be addressed and further investigated. One such is early menopause. Because of the deprivation of oestrogen after menopause, women are more vulnerable to bone loss than men, and women with early menopause are particularly at risk. Bone strength depends not only on bone mineral density but also on the size and structure of the bone. Therefore, instead of osteoporosis the wider term bone fragility can be used, indicating that the skeleton's susceptibility to trauma goes beyond low bone mass.

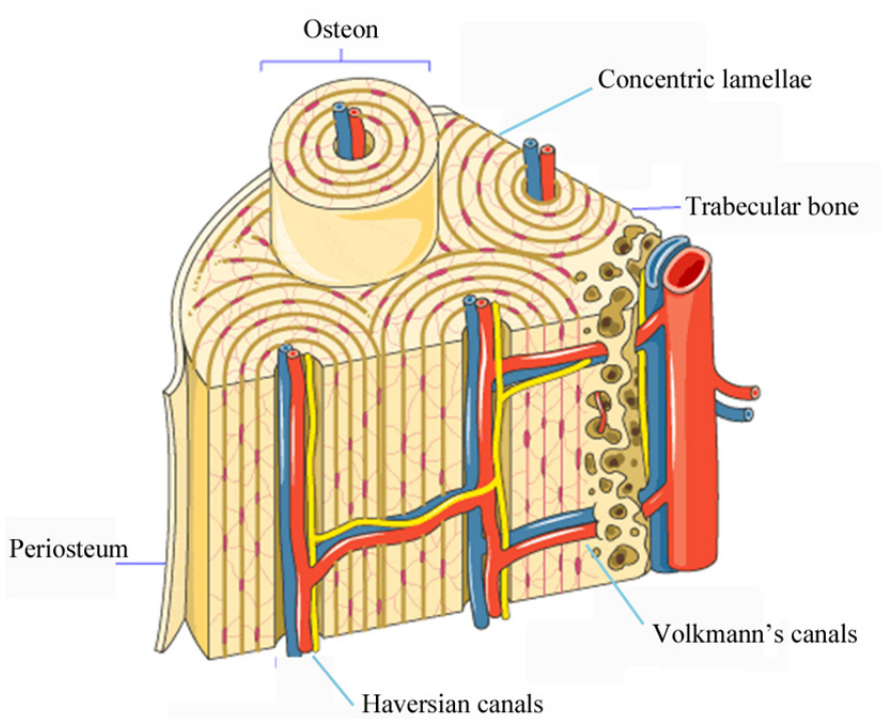
This thesis evaluates the long-term risk of bone fragility, fracture and mortality in women who have had an early menopause. We also investigate the long-term effect of physical activity in the postmenopausal period and the phenomenon of periosteal expansion with increasing age.

The skeleton and bone tissue

The principal purposes of the skeleton are three in number. The first is mechanical: to keep the body upright and provide rigid levers for the muscles to produce movement and speed. The second is to protect the vital organs and the haematopoietic tissues, and the third is to serve as a mineral reservoir, mainly for

calcium. In the first two aspects, the skeleton is divided into two structural units: the axial skeleton including skull, spine, sternum and ribs; and the appendicular skeleton including the tubular bones of the extremities. The material and structure of the skeleton are selected by evolution to balance the contradictory needs of strength and lightness, rigidity and flexibility. Saturating the bone tissue with mineral will increase its resistance to bending forces, but excessive stiffness will make the bone brittle, like glass. A too large proportion of collagen-weave and too little mineral will increase the elasticity when subjected to outer forces, but also risk plastic deformation of the bones.

Fig. 1.
Cortical bone structure and internal organisation.
Picture adapted with permission from the International Osteoporosis Federation.



The bone fabric is composed of collagen fibres surrounded by a matrix of calcium and phosphate in the form of hydroxy apatite crystals. The matrix constitutes the inorganic components which represent 70–75% of the bone mass; 20% are organic components of which 98% are collagen fibres and other proteins and 2% bone cells, and the remaining 5% of the bone tissue is water. There are three types of bone cells: 4–6% are large, multi-nucleated osteoblasts derived from mesenchymal stem cells and producing the hydroxy apatite matrix; 1–2% are bone-resorbing

osteoclasts derived from haematopoietic stem cells; and 90–95% are osteocytes, large stellar-formed cells whose extended network guards the integrity of the bone tissue and provides a mechano-sensor function which mediates the activity of osteoblasts and osteoclasts in response to external stimuli.

Morphologically, there are two types of bone tissue: trabecular or cancellous bone which constitutes 20–30% of the aggregated bone mass and cortical or compact bone making up approximately 75–80%. Trabecular bone is found in the vertebrae and the metaphyses of the long bones of the appendicular skeleton. It is a porous tissue consisting of trabeculae arranged in three-dimensional networks, producing maximal strength for minimal weight. Its porosity involves a large total surface area which makes the bone tissue more metabolically active, with a remodelling rate of 20–25% annually. This makes trabecular bone more susceptible to outer external stimuli; hormonal, pharmacological or mechanical, and it also here that postmenopausal bone loss is more pronounced [44, 52]. Consequently, trabecular bone is more prone to fracture than cortical bone but also heals more quickly.

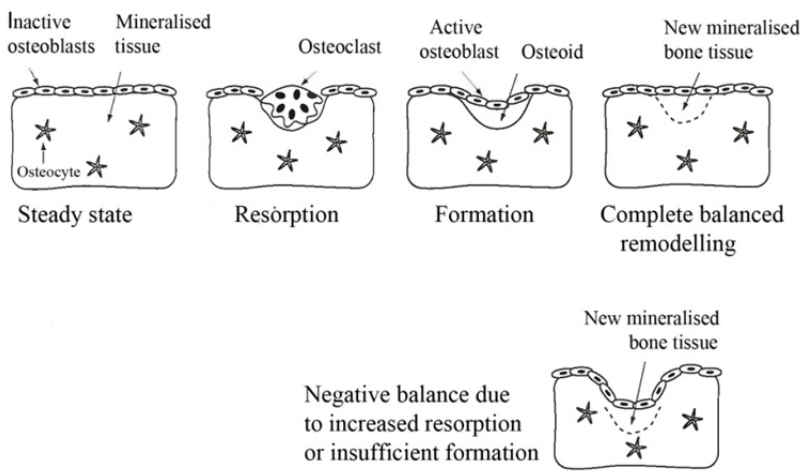
Cortical bone is found in the diaphyses of the long bones and also as a protective layer outside the trabecular bone. Its fundamental unit is the osteon, a structure with repeating layers, or lamellae, of compact bone tissue, surrounding the longitudinal Haversian canals where nerves and blood vessels run. The osteons are organised in an overlapping, brick-like construction to maximise resistance to cracks. The Haversian canals are connected with each other and with the periosteum by perpendicular Volkmann's canals. Osteocytes are contained in spaces in the bone matrix called lacunae. Cortical bone remodels with an annual rate of 3-5% and its hard structure makes it less vulnerable than trabecular bone but also means that fracture healing is slower.

Bone remodelling

Historically, bone was regarded as an inert tissue. By the early 20th century it was recognised that bone is built up by osteoblasts and eroded by osteoclasts. However, the cells were believed to act separately and independently of each other, before the dynamics of bone tissue were outlined by the American orthopaedic surgeon Harold Frost in 1963. Frost discovered and described the concept of basic multi-cellular units, BMUs [48, 111], which are clusters of osteoblasts, osteoclasts and their precursors functioning in one unit in the process called bone remodelling. This cellular machinery constructs bone during growth and reconstructs it in adulthood.

Bone is constantly remodelling, to adapt to mechanical forces, to repair micro-damage and to mobilise calcium from bone when serum levels are low. About 10% of the total bone tissue is remodelled annually, 20–25% in trabecular bone and 3–5% in cortical bone. Remodelling mainly takes place on the outer surface of trabecular bone and in the Haversian canals of cortical bone. The process starts with bone resorption as osteoclasts migrate from the bone marrow to the bone surface and remove bone to create a groove. After some three weeks, bone-forming osteoblasts are attracted to the resorption pit and the BMU is created. The osteoblasts fill the pit with collagen-containing matrix called osteoid which is later mineralised to bone (ossification). Some osteoblasts form the Haversian canals and others are entombed in small cavities in the bone matrix called lacunae and develop into osteocytes. The end result of each BMU in cortical bone is a new osteon. The whole remodelling process is complete after three to four months and should ideally not alter the dimensions and structure of the skeleton. Bone remodelling is also responsible for fracture healing and repair of micro-damage after normal physical activity, and responds to altered mechanical demands.

Fig. 2.
Schematic bone turnover at cellular level.
Adapted with permission from the Swedish Council of Technology Assessment in Health Care [2].



In the last decade, the role of RANKL (receptor activator of nuclear factor kappa-B ligand) as a principal mediator of bone remodelling was discovered [77, 154].

RANK ligand is today regarded as the central moderator in balancing osteoblast and osteoclast activity [118, 141], mediating the effects of e.g. oestrogens [84] and possibly vitamin D [129]. Via the RANKL system, the lifespan and activity of osteoblasts and osteoclasts are either promoted or inhibited, in turn altering the balance towards either bone formation or resorption, depending on the stimulating substance involved [118, 141]. Inhibiting the RANKL pathway decreases resorption and stimulating the RANKL pathway has the opposite effect [84].

During childhood and adolescence, when the skeleton is built up, bone formation is predominant over resorption. This *modelling* process is characterised by large increments in bone mass and an increase of the cortical thickness and periosteal diameter, i.e., the skeletal dimensions are altered too.

Biochemical markers of bone metabolism

Changes in bone matrix metabolism are reflected by biochemical bone turnover markers in serum. The most sensitive bone formation markers are osteocalcin and bone-specific alkaline phosphatases (ALP) produced by osteoblasts, whereas crosslinked C- (CTX) and N- (NTX) telopeptides of type I collagen are resorption markers. In postmenopausal osteoporotic women, elevated levels of bone resorption markers have been associated with increased fracture risk, independently of BMD. A combination of measurements of bone markers and BMD could thus help improve fracture risk estimates. Although the biological variability is large, bone markers could also be useful for monitoring anti-resorptive therapy as a more accessible and fast-responsive alternative to repeated BMD measurements [29, 55].

Endogenous factors affecting the skeleton

Bone metabolism is regulated mainly by hormonal substances and serum calcium levels. Among the naturally occurring substances in the body, oestrogen is probably the one with the strongest impact on bone tissue. Calcium homeostasis is also of great importance, since the skeleton is the body's calcium reservoir and the body will respond to low calcium levels in serum by releasing calcium from the skeleton through bone resorption. Calcium homeostasis is mainly regulated by interplay between vitamin D and parathyroid hormone, and to some extent calcitonin. To keep the bone tissue mineralised, an adequate supply of dietary

calcium is mandatory. This in turn relies on proper nutrition and exposure to sunlight.

Vitamin D is in effect a hormone synthesised in the skin when exposed to solar ultraviolet B radiation. Thereafter it is converted in two steps in the liver and the kidneys into its active form, calcitriol. There is also a dietary intake of vitamin D, especially in fatty foods from fish, dairy and liver, but this does not amount to the body's full need. Exposure to sunlight is required for maintaining vitamin D levels, which put people in the northern hemisphere at higher risk of deficiency. While lack of vitamin D probably is not a major cause of bone loss, it is essential for the development and growth of the adolescent skeleton. The main function of vitamin D is to enhance calcium absorption in the intestines and to stimulate the osteoblasts to mineralise the skeleton, the failure of which ultimately leading to rickets with soft, deformed bones in the growing skeleton, or osteomalacia in adults. The mineralisation is mediated through vitamin D receptors, enhancing osteoblast differentiation and inhibiting osteoclast activity, possibly mediated by the RANK ligand system, and vitamin D also stimulates the production of collagen and other proteins. Vitamin D regulates calcium homeostasis in an intricate interplay with PTH where low vitamin D levels lead to low serum calcium, in turn stimulating PTH to release calcium from the skeleton; low bone mass in patients with vitamin D deficiency is mediated by secondary parathyroidism through this mechanism [67, 129]. In addition to its direct effects on the skeleton, low vitamin D levels has been associated with fall-related factors such as inferior muscular strength, balance and gait speed [57].

Parathyroid hormone (PTH) is secreted by the parathyroid gland when serum calcium levels are low. PTH increases calcium levels by stimulating the osteoclasts to mobilise calcium from the skeleton, and the kidneys to increase calcium resorption and vitamin D activation [67]. Continuous increase in PTH, which is the normal physiological response to low calcium levels, consequently decreases bone mass. For unknown reasons and quite paradoxically, an intermittent supply of PTH instead has anabolic effects on the skeleton, promoting the deposition of bone on both surfaces of the cortex and also thickening the trabeculae [143]. This is used for pharmacological purposes and makes PTH the only anabolic anti-resorptive drug.

Calcitonin is another hormone affecting bone remodelling and calcium levels. It is synthesised in the parafollicular cells of the thyroid gland and acts as an antagonist to PTH – it inhibits bone resorption through direct effect on the osteoclasts and thereby lowers serum calcium levels.

Fig. 3.
Synthesis and activation of vitamin D.
Adapted with permission from the Swedish Council of Technology Assessment in Health Care [2].

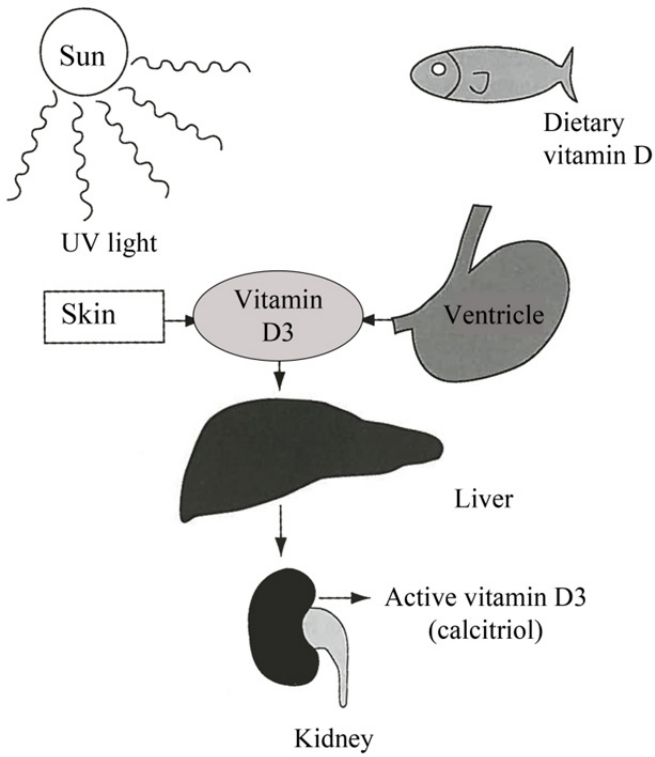
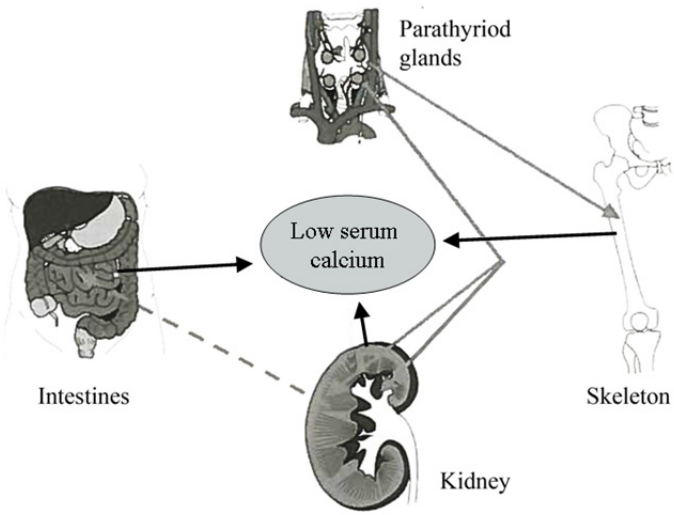


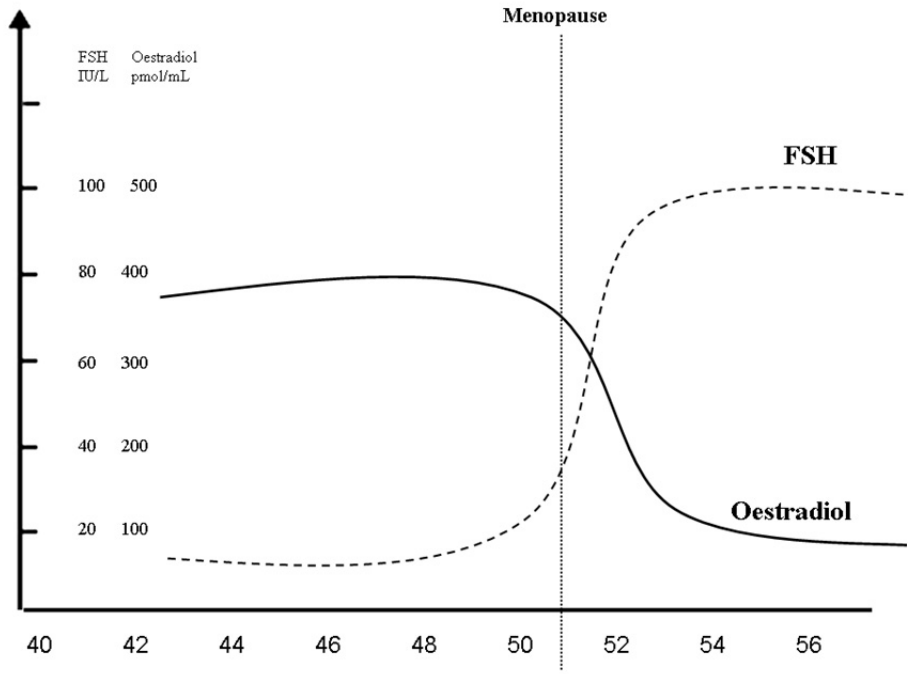
Fig. 4.
Schematic regulation of calcium homeostasis. Parathyroid hormone (PTH) increases calcium levels through osteoclastic activity on the skeleton, and increased calcium resorption in the kidneys. Calcitonin counteracts the osteoclastic effect of PTH. Vitamin D stimulates calcium absorption in the intestines and deposition in the skeleton.
Adapted with permission from the Swedish Council of Technology Assessment in Health Care [2].



Oestrogen is the collective name for a group of steroid compounds named for their role as the principal female sex hormones. Oestrogens exert strong effects on the skeleton and a number of tissues and organs in the body, which becomes obvious after the menopausal hormonal transition. During the fertile phase of life, the ovaries synthesise oestrogens from androgens in a cyclic pattern under the control of a feedback system involving follicle-stimulating hormone (FSH) and luteinising hormone (LH) from the pituitary gland. Oestrogens exist in three natural forms: oestradiol, oestrone and oestriol. Oestradiol is the predominant and most potent oestrogen during the fertile period. During the approximately 4–5-year-long perimenopausal period, decrease in hormone production in the ovaries occurs gradually and the menstrual cycles become irregular and longer. Markedly increased FSH levels can be measured during these transitional years but oestrogen levels remain normal or even slightly elevated until the last year pre-

menopause [117, 119, 151]. Oestrogen levels then wane rapidly but the decline evens out about one year after menopause and oestradiol levels stabilise [91, 119]. After the menopause, oestrogens are aromatised in the peripheral fat tissue from androgens produced in the adrenal gland. Oestrone is then the predominant form from which oestradiol is derived in small amounts [151].

Fig. 5.
Schematic figure of average oestrogen and FSH levels throughout the perimenopausal transition.



While its impact on bone accrual and bone loss is obvious, the precise mechanisms by which oestrogen acts on bone tissue have long been incompletely understood. The actions of oestrogen on bone are general, affecting several aspects of bone remodelling, and a number of mechanisms have been proposed. It has been generally considered for a long time that the basic role of oestrogen on bone is to stimulate bone formation and suppress resorption; already in 1940 Albright associated reduced bone formation with oestrogen deficiency [6]. Since the resorption phase of the bone remodelling process is much shorter than the formation phase, any acceleration of the process means that resorption will occur at a faster rate than bone formation and become predominant. Not until recent years has it been discovered that oestrogen effects seem to be mediated by the RANKL system [84] which regulates the balance between osteoblasts and

osteoclasts. Through its α-receptors [87], oestrogen inhibits RANKL and thus increases the lifespan and activity of the osteoblasts, and induces apoptosis of the osteoclasts [69, 84]. Postmenopausal oestrogen deficiency is associated with increased production of RANKL [42] and consequent increased osteoclast predominance and bone resorption. It is also believed that the decline in oestrogen leads to lower responsiveness to mechanical load, which reduces the bone formation effects of physical activity [13, 86]. On the structural level, experimental studies have indicated that oestrogen inhibits periosteal bone formation [163], and waning oestrogen levels post-menopause have been associated with increased endocortical bone resorption [51, 112]. This would be consistent with the oestrogen-driven bone accrual on the endosteal cortex seen in adolescent girls, and women's smaller bones compared to men [18, 140]. In addition to its direct effect on the skeleton, oestrogen has also been suggested to be associated with maintenance of vitamin D levels [125] and inhibition of the bone's responsiveness to PTH [33].

External factors affecting the skeleton

Mechanical load

One plausible cause of the increased prevalence of osteoporosis is a more sedentary lifestyle, as our modern way of living has gradually reduced the need for physical activity in everyday life. The skeleton adapts to the applied forces, and in the absence of mechanical load, bone strength will decrease. Already in 1892, the German anatomist-surgeon Julius Wolff stated in his theory, which was to become known as Wolff's law, that bone will adapt when subjected to external forces. Still today, the exact osteogenic mechanism is not known in detail but it is considered that mechanical forces exert strain on the skeleton which responds by forming bone tissue on the loaded site. Based on the mechano-stat theory developed by Frost [49], bone adaptation is stimulated by local strain. When mechanical forces exceed a certain level, which is probably genetically predetermined, micro-deformation is inflicted on the bone. This is detected by osteocytes, whose position and extended network serve them well in their mechano-sensory function. Osteocytes now enhance osteoblastic bone formation and inhibit osteoclastic bone resorption. [49, 50, 120, 130, 134, 161, 162]. The net result is local bone formation, which is site-specific – it has e.g. been shown in tennis players that bone mass in the dominant arm is higher than in the non-dominant arm [85]. The varying contour of the cortices also reflects adaptations to site-specific external loads, as e.g. the distal femoral neck is elliptical and wide in order to maximise resistance to bending forces, whereas the proximal femoral neck, which is subjected to

compressive forces, is more circular [143]. Dynamic, rapid, intermittent, high-impact load is more osteogenic than static load; i.e. short bouts of high-magnitude loading with periods of rest and diversely distributed strains on the bone stimulate bone adaptation more than long-duration, low-impact, monotonous loading [131, 162]. The anabolic effects on bone mass accrual and bone structure in the growing skeleton are well-documented and are most pronounced during early puberty, probably owing to the combination of an intense hormonal milieu and the rapid skeletal and muscular growth which itself increases mechanical stimulation of the skeleton. Consequently, pre- and early puberty can be regarded as a "window of opportunity" for optimising peak bone mass. The anabolic effects are further enhanced by a proper dietary calcium intake. [66, 81, 90].

Physical activity

While the effects of physical activity in adolescents [66, 81, 90], young adults [25, 95] and athletes [157] have been an intense field of research, the effects on bone structure in postmenopausal women have not been studied to the same extent. The majority of studies on all patient categories are short-term, and it has not been explored in detail whether the positive effects are maintained in adulthood nor if they could be associated with fracture prevention.

The effect of physical activity on areal BMD in postmenopausal women has been evaluated by dual energy X-ray absorptiometry (DXA) in a number of studies. The vast majority are designed as controlled exercise intervention programmes seldom exceeding two years and with increments in areal BMD of 1–3% [95, 147, 166]. In the few studies conducted with peripheral quantitative computed tomography (pQCT), similar short-term intervention programmes have conferred average effects of less than one per cent in volumetric BMD in both trabecular and cortical bone [114], the impact being somewhat more pronounced in the close menopausal period. No significant effects have been documented as regards bone geometry [114] or bone strength [105].

Also walking for preservation of bone mass in postmenopausal women has been estimated, with significant but modest effects on femoral neck BMD but not in the lumbar spine [96]. A substantial reduction in hip fracture incidence has been reported in postmenopausal women who walked at least 4 hours a week and did no other exercise [47]. Leisure physical activity has also been associated with reduced hip fracture risk in men [101]. However, these effects could also result from exercise-induced musculoskeletal effects that reduce the fall frequency. American public health recommendations have established a recommended minimum of 30 minutes of daily moderate physical activity five days a week or 20 minutes of high-intensity exercise three days a week for all healthy adults. [63]. These

recommendations have also been adopted by the Swedish National Institute of Public Health and the Swedish Society of Medicine.

Osteoporosis

The concept of osteoporosis was spawned already in 1824 by the English surgeon Sir Astley Cooper, who noted a relation between reduced bone mass and hip fractures in elderly [31]. The term osteoporosis itself is attributed to the French pathologist Jean Lobstein who coined the term in 1835, although probably referring to what today is known as osteogenesis imperfecta type 1 [89]. Senile osteoporosis was described in 1926 by the German Alwens [7] and menopausal osteoporosis was recognised in 1940 by the American endocrinologist Fuller Albright, who discovered the association between oestrogen deficiency and bone loss in postmenopausal women [6].

Historically, osteoporosis was a clinical diagnosis based on the presence of fragility fractures, and bone density was estimated visually from roentgenograms. The modern definition stipulates that osteoporosis is a chronic systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of the bone tissue, leading to enhanced bone fragility and as a consequence increased fracture risk [8]. Since DXA was introduced and widely accepted as the golden standard for bone densitometry, the World Health Organization (WHO) has defined operational criteria of osteoporosis based on DXA measurements of spine and hip in women [171], as listed below. These definitions consider only bone density and not the micro-architectural aspects of bone fragility. The WHO classifications are intended as guidelines for treatment of osteoporosis in postmenopausal women and do not apply to other patient categories.

BMD is reported not only in absolute values of g/cm² but also in terms of more easily interpreted T-scores and Z-scores which put the measured values into context.

The T-score represents standard deviations between the subject and average BMD in the healthy young adult population, and is the basis of the osteoporosis classification.

The Z-score represents standard deviations between the subject and average BMD in the age- matched population, or in the measured cohort. A Z-score lower than -2.0 could indicate the presence of underlying causes other than ageing-related or postmenopausal bone loss. In men and children, where there are no established operational definitions, the Z-score is a useful measure to evaluate osteoporosis and need for treatment.

Diagnostic category	Definition	BMD T-score
Normal	BMD not more than 1SD lower than the average in young adult individuals of the same sex and population	**–1 SD**
Osteopenia	BMD between 1 and 2.5 SD below the average in young adult individuals of the same sex and population	**1–2.5 SD**
Osteoporosis	BMD below 2.5 SD below the average in young adult individuals of the same sex and population	**–2.5 SD**
Manifest or established osteoporosis	BMD below 2.5 SD below the average in young adult individuals of the same sex and population plus at least one fragility fracture	**–2.5 SD**

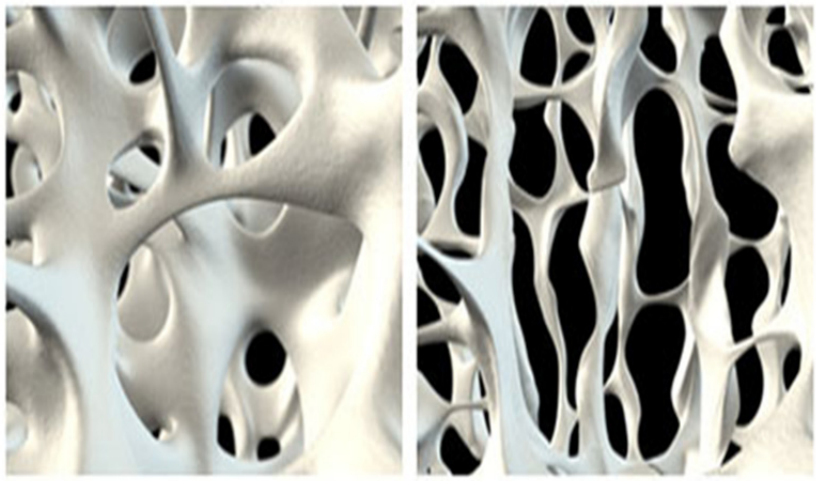
Table 1.
The operational osteoporosis classification according to the WHO.

Osteoporosis is classified into two forms: the primary osteoporosis which is idiopathic and includes both the early phase of rapid oestrogen-dependent postmenopausal bone loss in women, and the slower ageing-related bone loss seen in both men and women. The term secondary osteoporosis is used when a specific underlying cause is present, e.g. diseases such as coeliac disease, renal failure, anorexia nervosa, cancers, chronic obstructive pulmonary disease, hyperparathyroidism and Cushing's syndrome, or medication such as systemic glucocorticoid (cortisone) treatment.

The original description of menopausal and senile osteoporosis as separate conditions is today outdated and replaced by the current concept of a continuum

where the immediate postmenopausal phase of rapid bone loss passes on to a constant phase of slower bone loss throughout ageing.

Fig. 6.
Normal and osteoporotic bone.



Biomechanical aspects and bone strength

Along with the insight that the complexity of bone fragility goes beyond low BMD, the importance of the physical and biomechanical aspects of bone has gained increasing interest. The tendency among researchers to focus on the notions of bone fragility and bone strength reflects the fact that bone mass is not entirely responsible for fracture risk. Although BMD has been estimated to account for 60–80% of bone strength [20], the amount or density of bone mineral is only one of the bone's *material* properties; others are e.g. the integrity of the collagen fibres and the accumulation of micro-damage in the matrix. The skeleton's ability to withstand outer mechanical forces without fracturing also depends on its *macro-structural* or geometrical properties such as the periosteal diameter (bone width) and the thickness of the cortex; and the *micro-structural* or micro-architectural properties such as the number, thickness and network of the trabeculae, and the porosity and overlapping arrangement of the osteons in the cortices.

Material properties	**Structural properties**		
	Geometrical properties	**Micro-architectural properties**	
Degree of mineralisation	**Periosteal bone width**	**Trabecular**	**number** **thickness** **connectivity**
Quality and three-dimensional structure of collagen fibres	**Cortical thickness**	**Cortical**	**porosity** **overlapping structure of lamellae in the osteons**
Micro-damage accumulation			
Bone turnover rates			

Table 2.
List of material and structural properties of importance for bone strength.

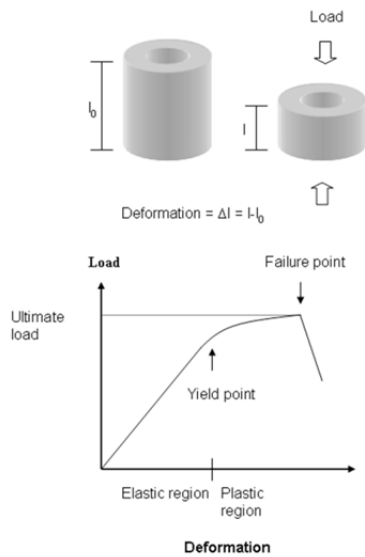
Reduced femoral neck width has been reported in men with hip fracture [144, 155], and smaller vertebrae in both men and women with vertebral fractures [39, 40, 144, 150]. Also longitudinal studies have shown a periosteal expansion of the femoral neck [164, 165] and the distal radius [5] but so far no association with increased fracture risk.

Men's larger bones make up a main cause for their higher bone strength and lower fracture incidence. Building large rather than compact bones is a functional adaptation to load – a denser bone than necessary would be energy-consuming to carry and maintain. Increasing bone width produces greater bone strength and is more cost-effective for the body [140].

When a bone is subjected to outer mechanical forces, or load, it will be deformed. If the load is only moderate, an elastic deformation will be caused, meaning that the bone will resume its original form when the load is released. If the load is heavier, the elastic capacity may be exceeded so that the so-called *yield point* is reached, causing a plastic deformation and micro-fractures of the bone. Even higher load will finally exceed the bone's plastic deformation limit or the *failure point*, and result in complete fracture. The failure point can be regarded as the ultimate limit of the bone strength. In mechanical calculations, load is converted to *stress*, referring to force per unit area, and relative deformation is described as *strain*. This means that a larger bone will be able to consume a higher stress than a smaller bone, which probably pertains to the compensatory mechanism of periosteal expansion with advancing age. The relationship between stress and strain before the yield point is reached represents a measure of the stiffness of the elastic material, or the *Young's modulus*.

Fig. 7.
Load-deformation test on a cortical bone segment. The unloaded segment, I_0, at upper left and the loaded segment, I, at upper right. The slope of the curve within the elastic region reflects the stiffness of the bone, and the area under the curve represents the energy required to reach the failure point where the bone will fracture.

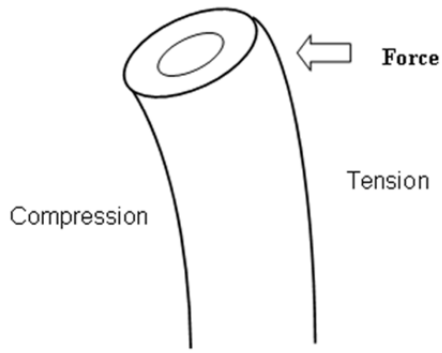
Picture by Henrik Ahlborg [3].



Load can be described in different terms, depending on the direction. Stress can be described as *compressive*, *tensile* or *shear*; most injuries involve a combination of these forces. Compressive stress is produced by axial load and the bone's resistance is decided by its cross-sectional area, or more simply: bone size. Tensile stress is produced by stretching forces and shear stress is produced by rotational load to the bone. Bending forces apply tensile stress on one side and compressive stress on the other. Theoretically, this would imply that the wider the bone, i.e. the farther away from the long axis the cortex is placed, the higher resistance to bending and shear forces.

Fig. 8. A bending force applied to a tubular bone results in tension on the nearest cortex and compression on the far cortex.

Picture by Henrik Ahlborg [3].

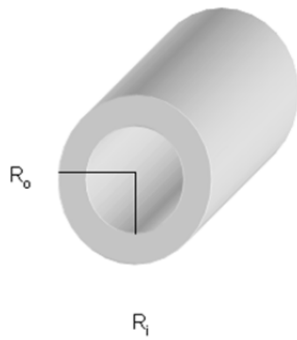


The *cross-sectional moment of inertia* and the *section modulus* are two key biomechanical parameters that represent the geometrical contribution to bone strength independently of material properties such as bone mass. These parameters describe the bone's resistance to bending forces and the cross-sectional moment of inertia has proven to correlate well with distal radius strength in cadaver studies [9]. Since the moment of inertia is proportional to the fourth power of the diameter of the radius, small increases in bone width generate large enhancements of bone strength. In this study, we have calculated a Strength Index by multiplying the section modulus by the cortical bone mineral density [68], thus taking both bone mass and skeletal structure into account. The Strength Index has been proven to correlate with mechanical strength in rat bones [46] but has to our knowledge never been evaluated with regard to fracture incidence in epidemiological studies. The bone's resistance to torsional forces is described as the polar moment of inertia and is also dependent on the distribution of the bone mass in relation to the bone's neutral axis, i.e. the periosteal and medullary width.

The micro-architecture of the bone is relevant to bone strength because cortical bone is more resistant to axial load, i.e. forces in the direction of the osteons, than perpendicular load and is also more resistant to compressive than tensile forces. Likewise, trabecular bone is more resistant to forces in the same direction as the majority of the trabeculae.

Fig. 9.
Geometrical basis and mathematical formulas of the biomechanical parameters cross-sectional moment of inertia, section modulus and polar moment of inertia. The calculations are based on the assumption that the bone is cylindrical. R_0 represents the outer radius (half the periosteal width) and the R_1 represents the inner radius (half the medullary width).

Picture by Henrik Ahlborg [3].



Cross-sectional moment of inertia = $(\pi/4)\ (R_o^4 - R_i^4)$
Polar moment of inertia = $(\pi/2)\ (R_o^4 - R_i^4)$
Section modulus = $((\pi/4)\ (R_o^4 - R_i^4))\ /\ R_o$

The concept of *periosteal apposition* is that the bone width increases with advancing age, as a compensatory response to decrease in BMD. A widening of the bone increases the cross-sectional moment of inertia and the section modulus, partly preserving bone strength. According to this concept, bone tissue is resorbed from the inner cortical surface, triggered off by the postmenopausal oestrogen deficiency, and deposited at the outer cortical (periosteal) surface. In this way, the cortex would become thinner but placed farther away from the longitudinal axis of the bone, increasing the moment of inertia [142]. This explanatory model is yet to be confirmed in longitudinal bone measurements.

Development of bone fragility – peak bone mass and bone loss

Although the structural properties are gaining increasing attention, bone mass is still regarded as the major determinant of bone fragility [20]. Principally, two essential determinants predispose the contribution of bone mass to bone fragility: the maximum amount of mineralised tissue reached at skeletal maturity, known as the peak bone mass, and the magnitude of bone loss after peak bone mass is attained. Peak bone mass is to a large extent decided by genetic or hereditary factors, according to estimations based on twin and family studies between 60 and 80% [43, 113, 145, 149]. The genetic contributions are complex, and it seems that a large number of genes are involved, all exerting modest effects. The most extensively investigated candidate genes are those expressing the vitamin D and oestrogen α receptors and collagen type 1α synthesis [61, 152].

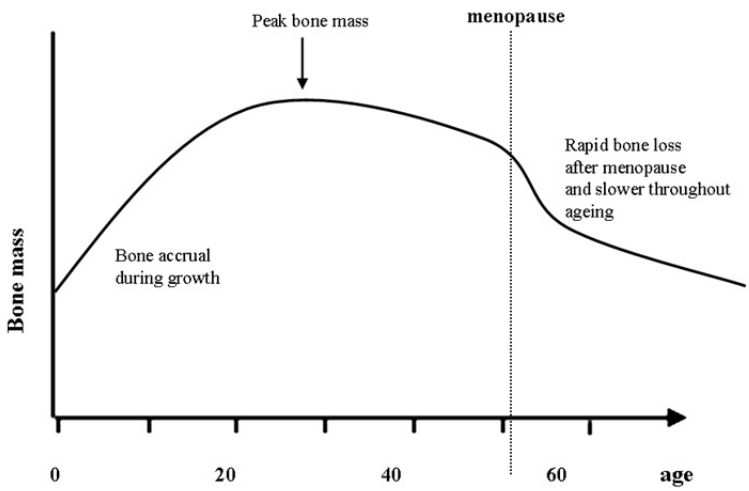
The remainder can be influenced by environmental factors during adolescence, particularly in the "window of opportunity" in pre- and early puberty when the fast-growing skeleton is the most susceptible to osteogenic load [18, 81, 140]. Physical activity, a proper dietary intake of calcium and proteins, and moderate smoking and alcohol habits are lifestyle factors that can help maximise bone mass accrual during this crucial period [18, 81]. The measured BMD increment is chiefly due to an increase in bone size; volumetric BMD remains largely constant. Men's higher BMD is established during puberty, as the later onset and longer duration of puberty in boys results in a longer window of opportunity and consequently larger bones [19, 140].

Peak bone mass is attained at different ages depending on skeletal site, and results have not been consistent. After completed growth bone mass has reached approximately 90% of its maximum, where after bone mineral may be accumulated in the hip and vertebrae up to the age of 20 or slightly longer. In the appendicular skeleton, the pattern is similar but bone mineral accrual seems prolonged and occurs even after the age of 30 [19, 97, 122, 137, 158]. In contrast to peak bone mass, bone loss seems mainly determined by environmental factors; according to recent reports hereditability accounts for 25–50% of the variance in bone loss in both the axial and appendicular skeleton [92, 146, 174]. Peak bone mass appears to be maintained up to mid-adulthood through bone remodelling, after which a slow but steady annual decrement in BMD of 0.5–1% begins. Bone loss seems to start at different times depending on the skeletal region; in the vertebrae already in the third decade of life and considerably later in the appendicular skeleton, according to some data not until perimenopause. The onset of menopause

signals a phase of accelerated bone loss amounting to 2–6% annually depending on skeletal site, mainly affecting trabecular bone. After 5–10 years, a steady state is reached and a continuous ageing-related bone loss of 1–2% annually prevails throughout life. [4, 30, 44, 45, 52, 58, 121, 128]

Postmenopausal bone fragility develops as the decreasing oestrogen stimulation of the skeleton increases the frequency of remodelling, resulting in an imbalance so that more bone is resorbed than formed at each remodelling site. This accelerates bone loss and damage to the micro-architectural structure. Trabeculae become fewer, thinner, perforated and disconnected, and cortical bone becomes thinner and porous. In the cortices, bone is resorbed mainly at the endocortical surfaces, partly compensated by periosteal bone formation [118, 140].

Fig. 10.
Schematic pattern of accrual and loss of bone mass in women.



Menopause

The menopause occurs as a natural part of a woman's ageing process and marks the end of the fertile phase of life. The word is derived from the Greek *men* (month) and *pausis* (cessation). Menopause is defined by the WHO as the permanent cessation of menstruation due to the permanent loss of follicular

activity [170], and can be established after one year of amenorrhoea. A catalogue of symptoms is associated with the menopause, all attributed to the oestrogen deficiency which has a universal impact on the female body. Best known are probably the vegetative or vasomotor symptoms such as hot flushes and night sweats. Also atrophic conditions of the urogenital tract are associated with the onset of menopause, and − arguably − cardiovascular disease and psychological effects [151]. Oestrogen is also one of the most potent actors in bone remodelling, its impact on the skeleton probably being even stronger than that on glandular tissues [115, 116], resulting in bone loss as one of the most devastating consequences of oestrogen deficiency.

In addition to lower peak bone mass, oestrogen deficiency is the main reason for the higher prevalence of osteoporosis in postmenopausal women compared to men of the same age. It has been estimated that after menopause, a woman loses on average 50% of her trabecular peak bone mass and 30% of her cortical peak bone mass [44]; almost half of these declines happen in the immediate postmenopausal period [124, 126]. All the classical osteoporotic fractures increase after menopause as a result of the bone loss [37], and women with early menopause are at particular risk. There is a body of evidence for the association between early menopause and increased risk of osteoporosis and fracture, although mainly based on cross-sectional or shorter longitudinal data [52].

Also increased mortality risk has been associated with women with early menopause. In a review from 2009 by Shuster et al., the overall morbidity and mortality from cardiovascular, neurological and psychiatric diseases was higher in women with early or premature menopause [148]. The chain of events can only be a matter of speculation; it is conceivable that early menopause is the cause of impaired health leading to an earlier death, through unfavourable oestrogen-dependent effects such as altered lipo-protein profiles [153]. In a meta-analysis by Salpeter [138] overall mortality was reduced by 39% in women who started hormone therapy (HRT) before age 60. Also in the Leisure World Cohort Study long-term HRT was associated with reduced total mortality [109], supporting the thesis that the increased mortality risk associated with early menopause is mediated by waning oestrogen levels. On the other hand, it could also be that underlying circumstances lead to both early menopause and premature death. In this case, early menopause would be the marker of risk and not the causal factor.

The mean age at menopause in the Western world is 51 [24, 60, 62, 99, 100] although the starting range is wide. *Early menopause* has gained wide recognition as a defined term, referring to menopause before age 45, and *premature menopause,* also known as premature ovarian failure (POF) or premature ovarian insufficiency, occurs before the age of 40. Approximately 10% of women in the Western world reach spontaneous menopause before the age of 45 [160, 167], and 1% before the age

of 40 [34]. Studies have identified some factors associated with early menopause, the most recognised of which are smoking [60, 102, 172] and low body weight [65, 172]. A special case is menopause induced by surgical removal of the ovaries. Although especially the onset of vasomotor symptoms is reportedly more abrupt, surgical menopause per se does not seem to be associated with an elevated risk of osteoporosis, fracture or mortality; rather this would depend on age at menopause [52, 148].

Risk factors for fracture

With the possible exception of vertebral deformities, low BMD alone cannot cause fracture; a fall or at least a minor trauma is also required. Bone fragility, fall risk and the type and energy of the injury are the three legs that constitute fracture risk. Low BMD is the most easily available measure of bone fragility and one of the strongest predictors of fragility fracture. Hence, measurement of BMD is perhaps the most useful tool for fracture prediction and it is documented that a 1 SD decrease in BMD implies twice the risk of fracture [74, 94]. Almost one quarter of all men and half of all women will develop osteoporosis and almost one half of all women will sustain an osteoporotic fracture [2, 79]. However, according to some data less than half of all patients with fragility fracture actually meet the diagnostic criteria of osteoporosis [139]. In fact, low BMD is only a *surrogate marker* of fracture alongside a list of other identified risk factors. While useful for fracture prediction on a group level, low BMD is less reliable when estimating the individual fracture risk. With advancing age, an increasing number of these predictors are likely to accumulate and reduce the contributions of low bone mass and early menopause to fracture risk. The prediction and prevention of osteoporotic fractures have been intensive fields of research in recent decades, and epidemiological data have identified a catalogue of risk factors for osteoporosis, falls and fracture.

Risk factors for osteoporosis [82]	Risk factors for falls [82]	Risk factors for hip fracture
old age	old age	old age [132]
female gender	female gender	female gender [132]
early or premature menopause		early menopause [52]
low body weight	tallness	low BMI [73]
sedentary lifestyle	gait and balance disorders	low physical activity [38, 73]
previous fracture	previous falls	previous fracture [38]
low dietary calcium intake	sensory impairments	low calcium intake [38, 73]
chronic illnesses/generally impaired health	medical co-morbidities	maternal history of (hip) fracture [38]
vitamin D deficiency		low sunlight exposure [73]
hyperparathyroidism		hyperparathyroidism [38]
glucocorticoid therapy	Use of benzodiazepines, antidepressants, diuretics, anti-hypertensive, anti-arrythmic, anti-seizure and sedative/hypnotic drugs	use of benzodiazepines or anticonvulsant drugs [38]
	musculoskeletal diseases	impaired neuromuscular function [38]
	visual impairment	poor visual acuity [38]
gastrointestinal disorders leading to malabsorption	postural hypotension	
	cognitive impairment	poor mental score [73]
alcohol abuse		high caffeine intake [38]
current smoking		
		late menarche [73]
		small bone size [144]

Table 3.
Risk factors for osteoporosis, falls and hip fracture.

Many risk factors overlap and are associated with a generally impaired health. Hip fracture is the most extensively studied fracture because of its devastating consequences for patients and society, and because it is easily evaluated in relation to DXA measurements of hip BMD.
In addition, environmental hazards, sometimes referred to as extrinsic risk factors, further increases the risk of falls. Among these are rugs, slippery surfaces, thresholds, chairs and beds without hand-rails, electrical cords on the floor, insufficient lighting, and improper footwear.

Because fracture risk assessment is complex and goes beyond the information of a single BMD measurement, the FRAX tool was conceived by the WHO and introduced in 2008 [78]. It estimates the fracture probability by taking a number of the above-mentioned risk factors into account, and it has been incorporated in clinical guidelines.

Fragility fractures

There are as yet no official or widely accepted definitions of the terms fragility fracture or osteoporotic fracture; different classifications have been used, based on anatomical site or mechanism of trauma. Almost all fractures become more common with advancing age and concomitantly decreasing bone mass, and it seems somewhat arbitrary which fractures should be attributed to bone fragility per se. Typical fractures associated with low-energy trauma in osteoporotic patients are fractures in the trabecular bone of the wrist, proximal humerus, ankle, tibia condyle, hip and pelvis, and vertebral compressions. Fractures of the hip, wrist and vertebrae have long been regarded as the quintessential osteoporotic fractures.

The Colles' fracture of the distal radius is the first osteoporotic fracture that begins to increase notably; the incidence rises rapidly after age 50, i.e. around the onset of menopause. In some countries, a plateau is seen in the seventh decade [32], but in Sweden the incidence seems to rise to steadily throughout ageing [17, 23].

Fig. 11a.
Fracture of the distal radius.

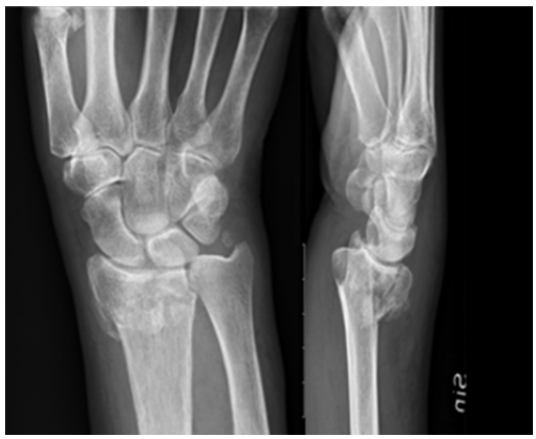
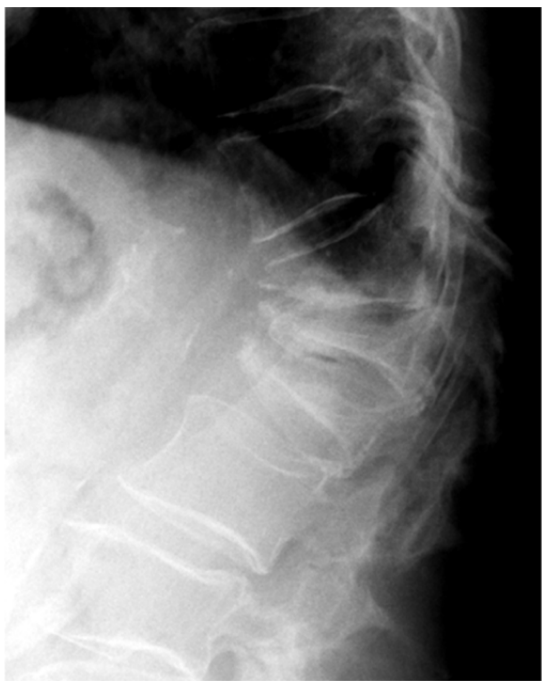


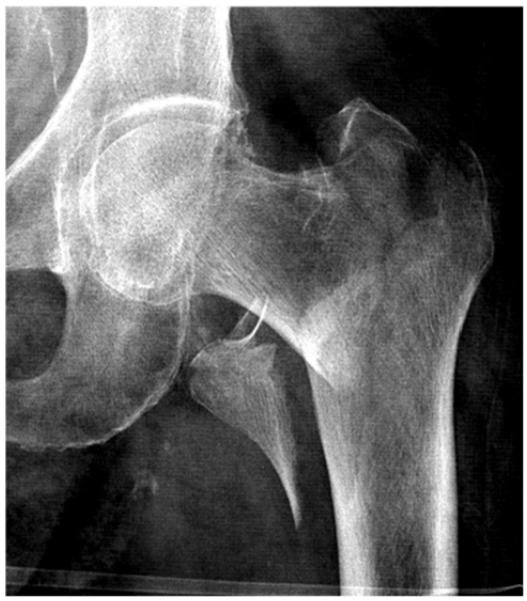
Fig. 11b.
Compression fractures of the vertebrae.



Vertebral compression fractures begin to rise rapidly after the age of 60 but are difficult to identify since the fracture seldom results from trauma, it is often asymptomatic and no standard classification exists. According to epidemiological

data from Hasserius et al., at least one vertebral deformity was found in 30% of 60–69-year-old women, 40% in 70–79-year-old women, and over 60% in women over 80 years of age [64]. Vertebral compression fractures typically result in pronounced kyphosis and reduced height.

Fig. 11c.
Fracture of the hip.



Hip fracture is the most common fracture after the age of 75 and the most devastating one, associated with the highest risk of co-morbidity and mortality and also the highest financial burden [1, 37]. Thus, hip fracture must be regarded as the ultimate consequence of bone fragility. The last century has seen a steady increase in hip fracture incidence in Sweden which now seems to have stabilised at a level of around 18,000 per year [132].

Fracture prevention and treatment of osteoporosis

The overall aim of all treatment strategies is to prevent fractures. This includes lifestyle recommendations regarding nutrition, alcohol intake, physical activity and smoking cessation. Strategies for fall prevention are essential for patients at particular risk. These include reduction of extrinsic risk factors for falls in the close environment, of medications that may cause dizziness and imbalance,

introduction of walking aids e.g. rollators, and implementation of exercise programmes to improve neuromuscular function. Special hip protector underwear has been developed, with a padding intended to absorb the energy from contusions to the trochanter.

Several fall-prevention intervention programmes have been presented; the most successful of these include multi-modal physical exercise focussed on balance, strength and endurance. Also moderation regarding multi-pharmacy and psychotropic drugs has proven valuable. [80]

The pharmacological treatment includes a number of medications, summarised below.

Vitamin D and calcium substitution is the basis of anti-resorptive therapy. A common dosage is 400–800 IE vitamin D + 500–1000 mg calcium per day, in addition to the current Swedish nutrition recommendations for postmenopausal women of 300–400 IE D-vitamin and 800 mg calcium – equivalent to approximately 8 slices of cheese or 0,7-0.8 litres of milk – per day, stipulated by the National Food Agency. During wintertime in Sweden, the sun is low and UVB light cannot penetrate the atmosphere, but in high summer 15 minutes in the sun a couple of days a week is regarded as sufficient to cover our needs. Vitamin D + calcium combination treatment has a documented effect on bone mass in osteoporotic patients [110] but can only marginally stimulate further mineral accrual in patients with adequate vitamin D levels. Meta-analyses have shown a preventive effect of combination treatment in adequate doses on both hip and other non-vertebral fractures [28, 67], especially in elderly institutionalised women [10, 28]. Vitamin D alone does not seem enough to reduce fracture risk [10, 28, 67]. Preventive vitamin D + calcium substitution can be recommended for patients with osteopenia or risk of developing osteoporosis.

Bisphosphonates were introduced in 1995 and is today the standard treatment in Sweden against established osteoporosis. The drug is a synthetic pyrophosphate analogue which builds complex with the hydroxy apatite in the bone. It inhibits osteoclast activity, both through direct cytotoxic effect on the osteoclasts and through its close affinity to the bone tissue, which blocks the osteoclasts.

Bisphosphonates are the most extensively studied pharmacological treatment of osteoporosis, with a documented effect on both bone mass preservation and fracture reduction [169]. There is evidence for positive effects on fracture prevention from five years of treatment and on bone mass from ten years' treatment. The effect on vertebral fractures seems solid with a reduction of 30–50%, whereas the documented effects on appendicular fractures have required large numbers needed to treat. Meta-analyses have shown BMD increments of 3–5% in the hip and 5–7% in the lumbar spine for alendronate and risedronate compared to placebo [35, 36].

Because bisphosphonates are retained in the bone tissue for at least ten years, the treatment can often be discontinued after five years in order to avoid accumulation or micro-fractures; bisphosphonates suppress not only bone resorption but also the normal physiologic remodelling process necessary for reconstruction and maintenance of the bone tissue. The consequence could be an adynamic bone with impaired repair of micro-damage which then would accumulate [108]. This could theoretically result in stress fractures, particularly the typical bilateral subtrochanteric insufficiency fracture which has been reported in patients taking bisphosphonates, although cases are very few in relation to the total exposure. Bisphosphonates are administered per os once a week or as an injection every three months and are combined with vitamin D + calcium supplementation. The patient is given strict instructions as to how to take the drug in order to maximise uptake and minimise adverse effects from the intestines.

Strontium ranelate has entered the market in recent years and is currently the third drug of choice in Sweden. Its mechanism is not entirely clear, but strontium acts similarly to calcium and is infiltrated in the bone tissue. It inhibits the osteoclasts but may also have some anabolic effects. There is documentation of reduced fracture incidence and BMD increases on parity with bisphosphonates, although no direct comparison studies exist [107, 123].

Recently, **denosumab** has also been introduced, a monoclonal antibody inhibiting the RANKL (receptor activator of nuclear factor kappa-B ligand) which is the common factor mediating osteoclast development. The few studies so far have indicated an effect of denosumab on BMD equal to bisphosphonates [22], although they have not been directly compared.

Hormone replacement therapy, HRT, are oestrogen preparations with a long tradition and well-documented solid effect on both bone mass in postmenopausal women [168] and fracture risk [133, 159]. However, HRT has been radically re-evaluated after the publication of two highly influential randomised controlled trials, Women's Health Initiative (WHI) [133] and Heart and Estrogen/progestin Replacement Study (HERS) [72], where oestrogen, contrary to previous belief, was reported to confer no protective effect and even increased the risk of cardiovascular disease [93]. In addition, HRT increases the risk of breast cancer and venous thrombo-embolism. Selective Estrogen Receptor Modulators (SERMs) were developed to block oestrogen-mediated breast proliferation while maintaining the bone-preserving effect; results showed protective effects on breast cancer but were not reinforcing as regards cardiovascular disease, stroke or thrombo-embolism [12]. The findings on the cardiovascular effects of HRT have been contentious, and in a meta-analysis by Salpeter [138] overall mortality was actually reduced by 39% in women who started hormone therapy before age 60. Also in the Leisure World Cohort Study, long-term HRT was associated with

reduced total mortality [109]. Reports indicate that bone is more sensitive to oestrogen than other tissues [115, 116], which suggests a potential breakpoint below which the skeleton could be stimulated without adverse effects. However, given the deterrent effects of the WHI and HERS studies and the growing number of alternatives for osteoporosis treatment [83], it seems unlikely that oestrogen preparations would regain their position as first drug of choice.

PTH preparations are the only pharmacological agent in regular use with anabolic effects on the skeleton. Beneficial effects are documented on BMD, bone size and micro-architecture and on fracture risk [59]. Treatment is administered with daily subcutaneous injections, is expensive and reserved for patients with manifest osteoporosis or an estimated high fracture risk e.g. due to secondary osteoporosis.

Calcitonin preparations are usually administered by injections, in cases with Paget's disease (osteitis deformans) or hypercalcaemia secondary to malignancies, but are currently not recommended for treatment of postmenopausal osteoporosis.

Bone densitometry

Bone densitometry is based on the principle of absorption of radiation in the bone mineral. The absorptive ability of calcium hydroxy apatite, which is much greater than that of soft tissues, means that radiation can be used for assessing bone density. Different estimates are used to describe the amount of bone mineral and a number of measurement techniques have been developed.

The first bone densitometer was constructed in the USA in the early 1960s and marked the beginning of the era of bone densitometry. Cameron and Sorenson described single-photon absorptiometry (SPA) in *Science* 1963 [26]. At the same time, Bo Nilsson was developing a similar apparatus at the Department of Orthopaedics at Malmö General Hospital. After Cameron and Sorenson's publication, Bo Nilsson travelled to the United States to acquire an Americium radiation source, allegedly kept it in his chest pocket during the journey home and manually carved out its proper size with a knife before inserting it in his equipment to complete his own SPA densitometer. Bo Nilsson described his methods in 1964 [106] and the Malmö General Hospital soon became a pioneer centre for bone densitometry, systematically refining its field of application.

Bone mass estimates

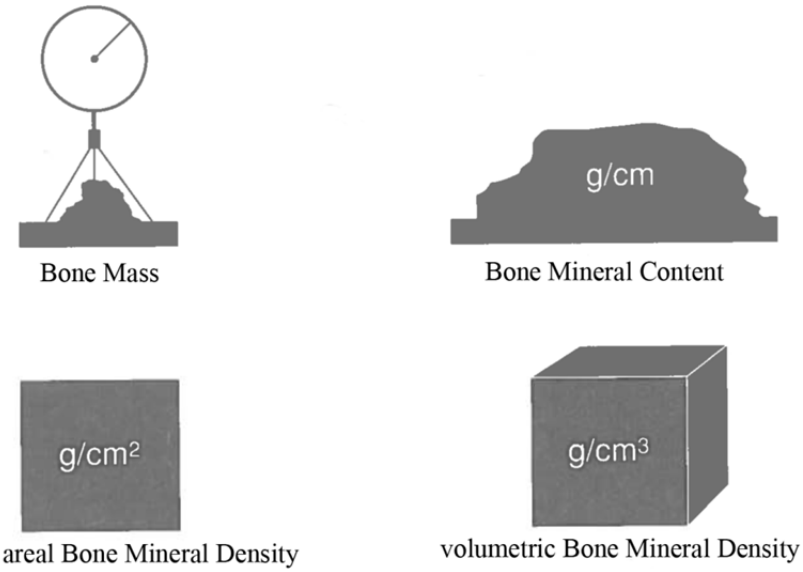
Bone mass is a non-specific and today somewhat antiquated term describing the amount of bone mineral. As bone densitometry gradually became more standardised – and especially with the implementation of DXA – the terminology has become more specified. The term bone mass is still used in general discussions, but in bone densitometry assessments the specific measure is preferred.

BMD − the standard measure produced by the bone densitometry equipment in use today, e.g. SPA, DXA and also pQCT, is in fact areal bone mineral density (aBMD), presented as g/cm^2. aBMD takes the periosteal diameter but not the depth of the measured bone into account. This is a source of error since a large bone will contain more bone mineral in the measured section than a small bone although the actual density is not necessarily higher; this will produce a falsely elevated aBMD value (fig. 15). A smaller bone will produce a lower aBMD value than a larger bone, although the actual density may be the same. This is a confounding factor that at least to some extent explains the lower BMD found in women compared to men.

BMC − bone mineral content, is a one-dimensional measure of the total amount of bone mineral in the scanned skeletal section, without regard to the width or depth of the measured bone. Consequently, BMC is likewise always higher in larger bones. However, for the same reason BMC is a more reliable estimate in longitudinal studies on changes in bone mass, since the results are not obscured by changes in bone size. BMC is presented as g/cm or just grams.

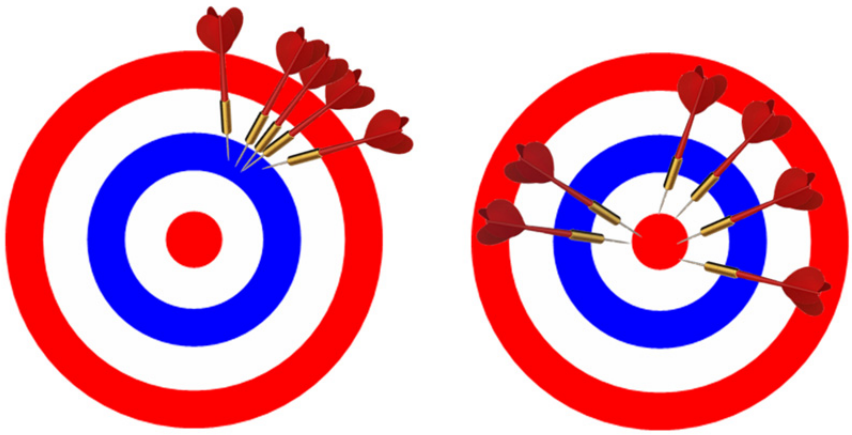
vBMD − volumetric bone mineral density (g/cm^3), sometimes referred to as bone mineral apparent density, is the true bone mineral density, measured three-dimensionally and taking both bone width and bone depth into account. While vBMD is the technically most exact estimate of bone density, it is in one respect a too narrow measure. Once the technique for assessing vBMD was developed, it proved that e.g. boys' higher bone mass compared to girls was conditioned by larger bones, not denser bone tissue –sex differences in adolescent vBMD are in fact modest [118, 140]. Today, only quantitative computed tomography (QCT) offers three-dimensional bone measurements.

Fig. 12.
Measures of bone mass and their geometrical basis.
Adapted with permission from the Swedish Council of Technology Assessment in Health Care [1].



Principally, bone densitometry is performed for two purposes: to measure the BMD e.g. for the diagnosis of osteoporosis; or to evaluate changes in bone mass, e.g. in longitudinal studies or for evaluation of osteoporosis treatment. The adequacy of bone densitometry methods is discussed in terms of *accuracy* and *precision*. Accuracy refers to the ability of the method to reproduce the true value of the subject, and is particularly important in cross-sectional studies when only one single measurement value is used. Precision is the degree to which the same value is obtained at repeated measurements of the same subject and is also called reproducibility. Depending on the purpose of the measurements, the preferable method may depend on whether its accuracy or precision is high. In longitudinal studies, a method with high precision is paramount if e.g. bone loss is to be measured reliably. Precision is affected by a number of factors, e.g. the phenomenon of counting statistics, calcifications and other degenerative abnormalities, imprecision caused by subject movements during the scan or the inability of the operator to relocate the exact region of interest. Precision is expressed either in standard deviations (SD) or as the coefficient of variation (SD in per cent).

Fig. 13.
Illustration of precision and accuracy in bone densitometry.



Bone densitometry techniques

Single-photon absorptiometry (SPA) uses ionising radiation of photons, i.e. gamma radiation and measures the bone two-dimensionally. It is applied on the appendicular skeleton, almost exclusively at the distal forearm, although in the early days the calcaneus, proximal tibia or distal femur were also used. The apparatus consists of a rectilinear scan with a gamma radiation source (usually Americium 241) moving across the bone and a scintillation detector following simultaneously. Because of the single energy beam, the scan cannot differentiate between absorption in soft tissue and bone and must therefore be applied to peripheral parts of the body with little soft tissue. The thickness of the soft tissue must also be constant around the perimeter of the measured limb, which is achieved by placing a rubber cuff around the forearm to compress the soft tissue. The cuff is water-filled and approximately of the same density as the soft tissue. Bone mineral density is calculated by multiplying the measured thickness of the

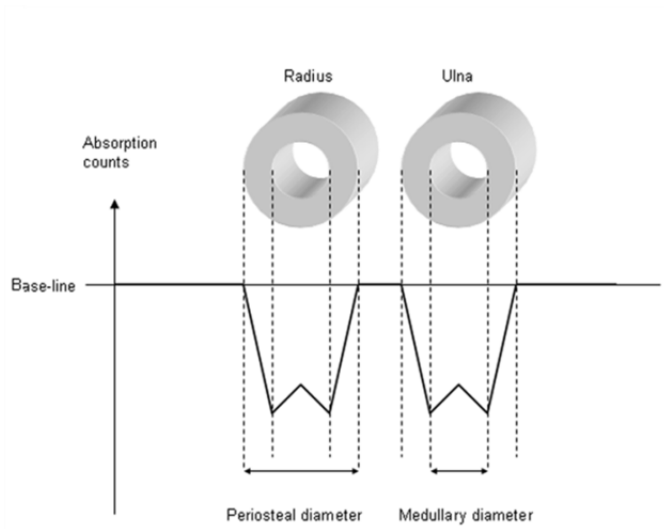
bone by the density of bone mineral (approximately 3000 mg/cm³). SPA also allows estimation of the width of the bone, using the graphical illustrations of the scan. The thickness of the cortex is calculated as the difference between the periosteal diameter (bone width) and the medullary diameters. The accuracy of the SPA method is approximately 9% [104] and the precision 1–2% [1, 27, 98, 103].

The peripheral measurement site at the distal forearm has several advantages. It is convenient for the subject and easily accessible. This facilitates the positioning of the subject which otherwise is a source of error. Furthermore, the distal forearm has relatively little soft tissue surrounding the bone, which makes the location suitable for a non-invasive method and the handling easier.

The original measure produced from SPA was presented as bone mineral content (BMC), but the unit used was g/cm^2. With the standardisation of terminology in the 1990s, the estimate was renamed bone mineral density (BMD), while the unit was kept the same. BMD is the adequate term since the measurement is two-dimensional and BMC today refers to one-dimensional bone measures.

Fig. 14.

The graphical representation of the output of the SPA scan. The absorption counts indicate how much radiation passes through the tissues, i.e. the radiation that is not absorbed. The baseline represents attenuation in the cuff and soft tissues, which is subtracted when calculating the density of the bone mineral. The trace also marks the contours of the bones and thus allows estimation of the total width, medullary width and cortical thickness.
Picture by Henrik Ahlborg [3].

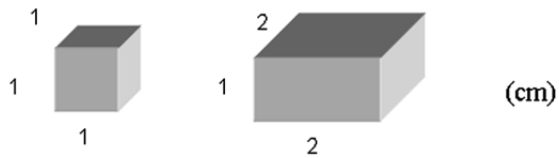


Dual energy X-ray Absorptiometry (DXA) was introduced in 1987 and has become the standard bone densitometry method. In 1994 the WHO established the criteria of osteopenia and osteoporosis as standard deviation change from reference BMD values in young healthy individuals (T-values), based on DXA measurements. In absolute numbers, these cut-off values for osteoporosis are 0.706 g/cm^2 for hip BMD and 0.907 g/cm^2 for lumbar spine BMD. Often, the standardised T- and Z-scores are used to allow comparison with reference populations. Hip and lumbar spine are the most common measurement sites but also whole-body scans are possible, including evaluations of muscle and fat tissue. The technique uses two low-dose X-ray beams of different energy levels where the low-energy beam is absorbed only in soft tissue and the high-energy beam is absorbed in both bone and soft tissue. Subtraction of the low-energy scan values provides estimates of BMC and aBMD. DXA measures the amount of bone mineral per unit area (aBMD) of a section of bone – it is two-dimensional and does not take the size and shape of the bone into account, which is its main limitation. An algorithm called Hip Strength Analysis (HSA) was developed by Beck et al. [15] and Yoshikawa et al. [173] to estimate femoral neck strength variables such as cross-sectional moment of inertia and section modulus by approximating the three-dimensional geometry from the antero-posterior image of the DXA scan. DXA measurements are afflicted with some confounders since degenerative changes on the vertebrae such as compressions, osteophytes and decreased disc height, vascular calcifications, and surgical implants may produce a falsely elevated value. In spite of its limitations, DXA is today widely implemented to evaluate bone health and diagnose osteoporosis, and is robustly associated with fracture risk [74]. In addition, basically all pharmacological treatments are developed and evaluated in relation to bone mass measurements by DXA. The precision of DXA ranges from 1–3% and the accuracy 3–9%, depending on measurement site [2].

Peripheral quantitative computed tomography (pQCT) is the most recent contribution to the arsenal of bone densitometry equipment. QCT models have also been used previously but involve high radiation doses at central parts of the body. The pQCT technique was developed to be more easily manageable and more favourable as regards radiation, since the measurement sites are the forearm and lower leg, distant from the vital organs. The QCT methods provide three-dimensional measures of BMD and bone geometry and distinguish between cortical and trabecular bone. Total bone area and the cortical, trabecular and medullary areas are measured, whereas periosteal diameter and cortical thickness are approximated by assuming the bone to be cylindrical. Muscle and fat tissue density is also measured. With the recent introduction of high-resolution pQCT systems (HR-QCT), even more refined assessments are possible with estimations

of the micro-architectural properties such as trabecular size and number. The precision is less than 1% for pQCT and 0.3–3.9% for high-resolution pQCT [135].

Fig. 15.
Effects of bone size on bone mineral measures in two 1 cm sections of bone. The bones have the same true BMD, but aBMD becomes double in the larger bone because of the inability of two-dimensional techniques to capture differences in bone size
Picture by Henrik Ahlborg [3].



True bone mineral density (g/cm^3)	1	1
Projected area (cm^2)	1	2
Volume (cm^3)	1	4
Bone mineral content (g/cm)	1	4
Bone mineral density (g/cm^2)	1	2

Aims of the study

The overall aim of this study was to prospectively evaluate risk factors for bone fragility and fractures in postmenopausal women, with particular attention to early menopause, physical activity and changes in bone density and bone size.

Our specific aims were:

To estimate whether menopause before age 47 affects the risk of osteoporosis, fracture and mortality also in a long-term perspective.

To identify independent risk factors for fracture risk in postmenopausal women.

To evaluate whether menopause before age 47 is an independent predictor of fracture and mortality or if its impact is mediated by other factors related to menopause.

To evaluate whether bone loss is affected by moderate physical activity in a long-term perspective in postmenopausal women.

To evaluate whether bone size and bone structure are affected by moderate physical activity in a long-term perspective in postmenopausal women.

To assess rates of bone loss in different time periods in relation to menopause.

To assess whether the periosteal expansion associated with the bone loss exists only in the first postmenopausal decade or if it continues in a longer perspective.

To assess whether mean estradiol levels or changes in estradiol levels could be correlated to bone loss and changes in bone size in the early postmenopausal period.

Hypotheses

Menopause before the age of 47 affects bone mineral density, fracture risk and mortality in a long-term perspective.

Menopause before the age of 47 is an independent risk factor for fracture and mortality.

Moderate physical activity in the postmenopausal period is associated with reduced bone loss and increased bone width.

Bone loss is highest during the eight years immediately following menopause, and remains constant thereafter.

Periosteal expansion occurs as long as bone loss occurs all throughout ageing.

Bone loss and periosteal expansion after menopause are both associated with a decline in serum oestradiol levels.

Material and Methods

The Malmö Perimenopausal Project was initiated in 1977 by the professors Bo Nilsson and Olof Johnell as a collaboration between the Departments of Orthopaedics and Gynaecology at the then Malmö General Hospital. The original aim was to study changes in hormone levels and bone mass during the menopausal transition. As the studied women had left the perimenopausal stage, the endocrine assessments ended, whereas the study continued at the Orthopaedic Department with repeated bone measurements. This resulted in a prospective observational population-based cohort study of unique length.

Subjects

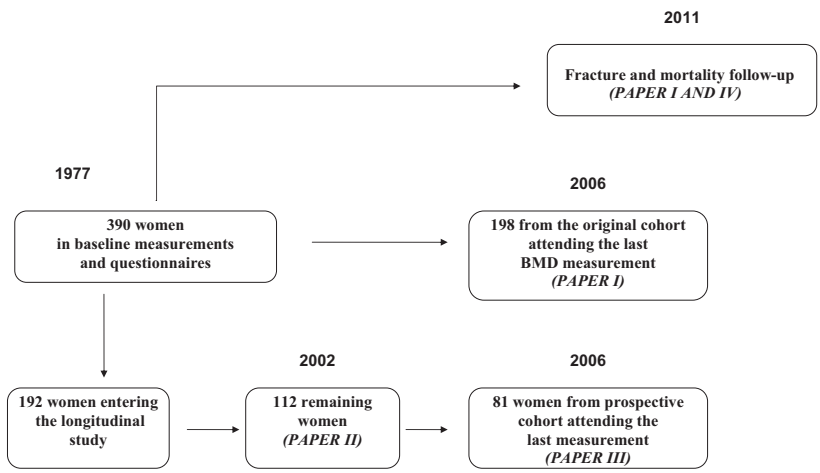
The studied population consisted of 395 women, all born in the latter half of 1929 and recruited from the populations registers of the city of Malmö, Sweden. When the first measurements began in 1977, the women were 48 years old. At baseline, general health and lifestyle parameters were noted by questionnaires and personal interviews. Anthropometric measurements were taken and forearm bone mass was measured with SPA.

Of the original 395 women, five were later omitted since their baseline measurements could not be retrieved, leaving 390 women to be included (**Papers I and IV**). At baseline, 109 women were postmenopausal, 45 declined continuous participation in repeated measurements and 49 were not eligible because of irregular menstruations, or interfering medication or diseases. This left 192 women who entered the longitudinal study. In the first five years, 17 women were excluded because of relocation or surgical menopause, 4 because of technical measurement errors and 15 because of perimenopausal HRT. In the ensuing 20 years, further 36 women were lost to follow-up owing to death, relocation or withdrawal for medical or personal reasons. In 2002, 120 women attended the 11th measurement at the age of 72, and 112 subjects with technically sufficient SPA measurements and complete questionnaires were included in **Paper II.** At the 12th and last measurement at age 76, 100 women participated. Twelve of these had

insufficient SPA measurements, and 7 had diseases or medications interfering with bone metabolism, leaving 81 women to be included in **Paper III.**

During the first twenty years of the study, bone measurements were done on average every second year. In 2001, at age 72, all original study subjects who were still alive and could be located were invited to a follow-up measurement. This included bone density measurements with DXA and spine radiograms in addition to SPA, anthropometric measurements, and questionnaires. These measurements were repeated at the 12th and last measurement series in 2005–07.

Fig. 16.
Flowchart of participants from 1977 to 2011.



Determination of menopausal status and age

In **Papers I** and **IV**, the 390 women were divided into categories according to menopausal status at study start. All women were 48 years old at study start, and to be certain to observe the WHO criteria that requires 12 months of amenorrhoea, we counted one year backwards so that age 47 became the cut-off value. This

resulted in one group of 61 women with menopause before age 47 and one group of 329 women with menopause at age 47 or later.

In the cohorts in **Paper II** and **III**, all women were premenopausal at study start and followed across their natural menopause which could be determined to the exact day by means of hormone measurements and questionnaire information on bleeding patterns.

Fracture ascertainment

We identified fractures sustained by the 390 women through repeated searches of hospital registrations and digitised databases, from age 48 until death, relocation or until the end-point date 30 September 2011 (age 82). The fracture registration period thus spanned 34 years, and since the incident fractures were objectively registered through their hospital attendances all women could be included, even those who declined follow-up BMD measurements. The fracture registration system at the department has been thoroughly evaluated and used in numerous epidemiological fracture studies [16, 53, 76]. All patients in the Malmö region attend the same trauma unit as there is only one emergency department in the city, and all radiographic examinations were routinely examined by two radiologists and then registered and indexed. Radiographs and reports have been kept on file for each subject since the beginning of the last century. Residents of Malmö who sustain fractures in other geographical regions are referred to the Orthopaedic Department of Skåne University Hospital in Malmö for follow-up. Fewer than three per cent of all fracture patients in the city visit a private physician and most of these cases are minor fractures not necessitating treatment, such as non-dislocated digit fractures [16, 53, 76] . Classified fractures are verified by the radiologists' original reports. Fragility fractures were defined as low-energy fractures (a fall on the same level) of the wrist, proximal humerus, spine, hip, pelvis, tibia condyle and ankle. Non-clinical asymptomatic vertebral fractures and non-osteoporotic high-energy fractures were not included in our calculations. Nine participants who had relocated to other regions in Sweden were telephoned and asked whether they had sustained any fracture. If fractures were reported, they were verified through case reports acquired from the respective hospital. In 11 women, fracture records could not be obtained as these women had either died after having relocated or could not be located. In these cases, we used the relocation date as the end-point data in the risk calculations.

Mortality registration

Mortality data during the 34-year follow-up period were obtained from the national population registers. At regular intervals, data on the number of deceased and death dates were acquired from the national population authorities. These data were complete in all but 9 cases; these were subjects who had left the study and moved abroad. Information on cause of death was not obtained; this is not recorded by the population registers and we did not have ethical approval to gather such data.

Bone measurements

Bone measurements were done by single-photon absorptiometry (SPA), which today is an outdated method, but it was the best one available in 1977. However, SPA was suitable for our longitudinal study design because of its high precision, low radiation doses and because estimation of bone structure is also possible. We used the method described by Nauclér et al., where bone is measured at one mainly trabecular site 1 cm proximally of the ulnar styloid process, and at one mainly cortical site 6 cm proximally of the ulnar styloid process [103]. The radii and ulnae of both arms were measured, and one average value from all four bones was used. The apparatus constructed by Professor Bo Nilsson in 1964 was used for all bone measurements with replacement only of the radiation source in 1980. The same anatomical site was used at all measurements and the same technician analysed all data. In the papers presented in this thesis, only data from the proximal site were used. This is for reasons of technical quality, since the ageing-related attrition is more pronounced on trabecular bone, which makes edge detection less reliable. Thus it is more difficult to obtain measurements of sufficient quality with increasing age. Assessments of a standardised phantom at least every second week were done all through the study period and did not detect any long-term drift of the densitometer. Since the radiation source was replaced in 1980, all bone mass measurements thereafter were recalculated with a correction factor provided from the phantom measurement data.

The precision of our SPA measurements was monitored with repeated measurements of a standardised phantom at least every second week. The week-to-week variation over one year was found to be just below 1% (coefficient of variation), which can be explained by the stochastic nature (the intrinsic random variation) of radiation and by the difficulty in reproducing the exact positioning of

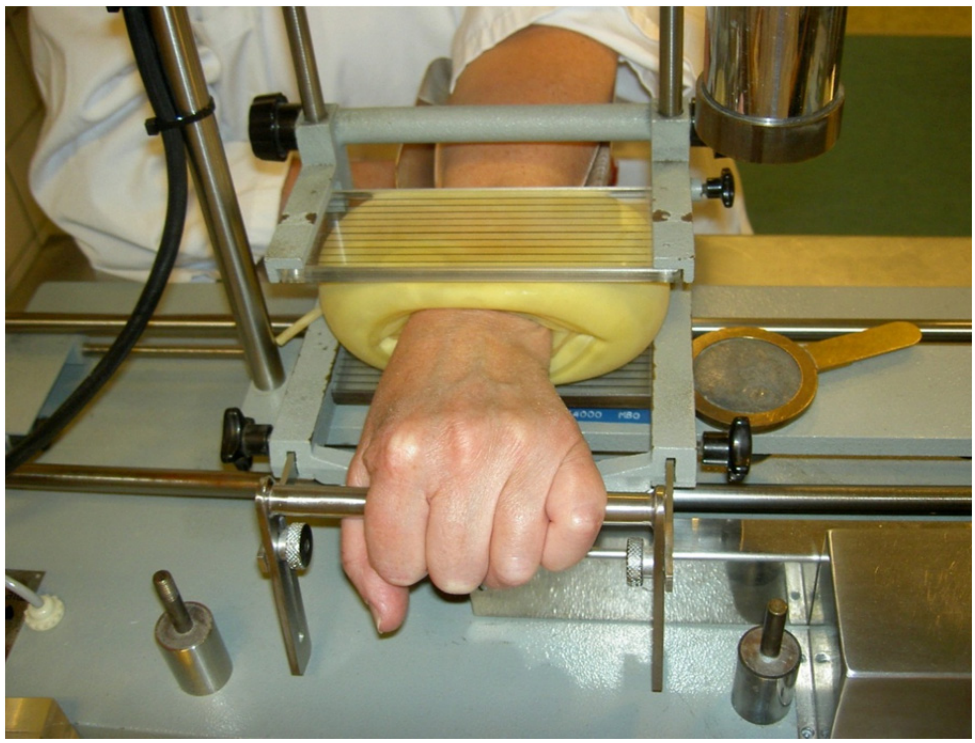
the subjects. The precision measured as the year-to-year variation between age 48 and age 72 was 1.6% for BMD and 1.8% for bone width, based on the phantom data. The precision of the method was also assessed as 20 subjects were measured on two different occasions not more than a month apart. This *in vivo* coefficient of variation was 4%. Linear regression equations of the phantom data showed that the long-term drift of the densitometer was only 0.1% per year (95% CI –0.2, 0.4) for BMD and 0.08% per year (95% CI –0.01, 0.17) for bone width. [3]

The graphical illustration of the scan marks off the outer and inner edges of the cortex, provided that the technical quality is sufficient. This allows estimating the periosteal diameter (total bone width) and the medullary width, and the cortical thickness is the difference between the two. The estimate of cortical thickness from the graph of the scan had a reproducibility of 8% [103]. The total cross-sectional area, the medullary area and the cortical area can be calculated by assuming the bone to be cylindrical [area = $(\text{diameter})^2 \cdot \pi/4$]. The densitometer measures the areal bone mineral density (aBMD) as mg per cm². The bone mineral content (BMC) in mg/cm was calculated as the aBMD divided by bone width. Volumetric BMD or bone mineral apparent density (BMAD) in mg/cm³ was calculated as bone mineral content per cortical area. The cross-sectional moment of inertia was calculated as [$(\text{periosteal diameter}/2)^4 - (\text{medullary diameter}/2)^4] \cdot \pi/4$ and the section modulus as [cross-sectional moment of inertia / (periosteal diameter/2)]. A Strength Index, taking both bone mass and skeletal structure into account, was calculated as the product of the section modulus and the bone mineral apparent density [68].

It can be added that originally, the estimate produced from SPA was denoted bone mineral content (BMC), but the unit used was g/cm^2. As bone densitometry became more standardised, especially with the implementation of DXA in the 1990s, the measure from SPA was changed to bone mineral density (BMD), and the unit was kept the same. BMD is the adequate term since the measurement is two-dimensional and BMC today refers to one-dimensional bone measures.

At age 72 and 77, BMD (g/cm^2) was also measured by dual energy X-ray absorptiometry (DXA; GE Lunar Corp., Madison, Wisconsin, USA; Lunar Prodigy®) in the hip and lumbar spine and was used for the osteoporosis diagnosis in **Paper 1**.

Fig. 17.
Bone measurement in the SPA apparatus.



Endocrinological measurements

The women who were still premenopausal, i.e. had cyclic although not necessarily regular bleedings (at least four episodes during the last 6 months), were invited to enter a prospective perimenopausal study. The purpose was to assess changes in endocrine levels during the menopausal transition and to determine age at menopause as exactly as possible. Menopause was defined according to the criteria established by the WHO [170]. Thus, the onset of menopause was determined retrospectively, after a period of 12 months of spontaneous amenorrhoea along with elevated levels of follicle-stimulating hormone (FSH).

Blood samples were drawn every 3 months during the first year, then every 6 months until one year after menopause, and thereafter every 12 months up to ten years post-menopause. Serum levels of luteinising hormone (LH) and follicle-stimulating hormone (FSH) were measured with double antibody

radioimmunoassay technique and started to increase five years before menopause. LH culminated within the first postmenopausal year and FSH 2–3 years post-menopause. Thereafter, both gonadotropins declined gradually. Serum oestrogen levels started to decrease during the 6-month perimenopausal period, more marked in oestradiol than in oestrone. In the following three years, the declines were moderate and parallel in both. Mean values 3.5 years post-menopause were 102 pmol/l for oestradiol and 148 pmol/l for oestrone. No decrease in oestrogen levels were found 3–8 years post-menopause [119].

Questionnaires

At baseline and at all follow-up measurements, general health and lifestyle data were registered through questionnaires and personal interviews. These included data on amounts of physical activity, nutritional intake, number of children, smoking, current and previous medication, and diseases.

At age 72, a detailed questionnaire on habits of physical activity was used. The women were asked to specify hours per week of everyday, general physical activity such as walking, cycling, gardening, swimming and scheduled physical exercise at the time of their menopause, five, ten and fifteen years after menopause, and currently. These data were used for **Paper II.**

Anthropometric measurements

Height and body weight were assessed at each follow-up examination with standard manual scales and weight meters. Grip strength for each hand was measured at baseline and at the two last measurements with a Martin vigorometer (Heinrich C. Ulrich, Werkstätten für Medizinmechanik, Ulm-Donau, Germany), and an average value of three attempts was noted.

Statistical methods

All statistical processing was done with STATISTICA software, version 7.1

In all papers, descriptive data are presented as means with 95 confidence intervals.

In **Papers I and IV**, group comparisons were done with student's *t* test between means in numeric variables and chi-squared tests in binary variables. In **Paper I**,

comparisons of the outcome variables – fracture and mortality – were made by calculating risk ratios as incidence per 10^3 person years and rate ratios by dividing the group incidences, and Kaplan-Meier survival curves presented with log-rank tests. Risk of osteoporosis was calculated with chi-squared tests. In **Paper IV**, univariate and multivariate survival analyses were done with Cox's proportional hazard regressions when calculating risk ratios for fracture and mortality.

In **Papers II and III**, annual percentual changes in bone mass and bone structure variables during different time periods were calculated for each woman as the ratio of the slope fitted to each woman's repeated measurements divided by the baseline value. In **Paper II**, analysis of variance (ANOVA) was used for estimating group differences in changes over time in e.g. bone loss, and when significant, confounders were adjusted for with analysis of co-variance (ANCOVA). Chi-squared tests were used for estimating group differences in binary variables and fracture rates per 10^3 and risk ratios for fracture were calculated.

In Paper III, linear regression equations were used for examining the association between variables.

Summary of papers

***Paper I*:** Early Menopause and Risk of Osteoporosis, Fracture and Mortality:

A 34-Year Prospective Observational Study in 390 Women

Introduction: Early menopause is a recognised risk factor for osteoporosis and fracture risk. This notion is based mainly on cross-sectional and short-term studies with a retrospective definition of age at menopause. Furthermore, some authors suggest that the influence of early menopause fades after the age of 70.

Aim: To determine whether the risks of osteoporosis, fragility fracture and mortality associated with early menopause prevail in a long-term perspective.

Methods: 390 women were studied from age 48, menopausal status was noted at baseline with age 47 as threshold for early menopause, and fractures and mortality were registered until age 82. BMD was measured at age 77.

Results: Women with menopause before age 47 had a risk ratio of 1.83 (95% CI 1.22, 2.74) for osteoporosis at age 77, a risk ratio of 1.68 (1.05, 2.57) for fragility fracture and a mortality risk of 1.59 (1.04, 2.36).

Conclusions: Menopause before age 47 is associated with increased risk of mortality, of sustaining fragility fractures, and of osteoporosis at age 77.

***Paper II*:** Physical activity reduces bone loss in the distal forearm in postmenopausal women – A 25-year prospective study

Introduction: Numerous studies have shown that bone mass in postmenopausal women can be slightly increased by physical exercise. However, the vast majority have been intervention studies with high-intensity training programmes and a duration of 1–3 years. Effects of physical activity on bone structure have not been evaluated.

Aim: To determine whether moderate everyday physical activity could be associated with reduced postmenopausal bone loss also in the long-term perspective and with changes in bone structure.

Methods: Bone mass and bone structure of the distal forearm were evaluated in 91 moderately physically active and 21 inactive women with repeated SPA

measurements starting at menopause and continuing for 25 years. A strength index was calculated, taking both bone mass and bone structure into account.

Results: The mean annual loss in BMC was 1.2% (95% CI 1.1, 1.3) in the physically active and 1.6% (1.3, 1.8) in the inactive women (after adjustment for menopausal age p=0.02). The mean annual decline in the strength index was 0.7% (0.6, 0.8) in the physically active and 1.2% (0.8, 1.5) in the inactive women (p=0.004). At age 77, BMC was 0.5 SD (0.1, 1.0) higher in the physically active women. No group differences in changes in bone structure were found.

Conclusions: Physical activity is also in a long-term perspective associated with reduced postmenopausal bone loss, but does not appear to affect bone structure.

Paper III: Changes in Forearm Bone Mass and Bone Size after Menopause – a Mean 24-Year Prospective Study

Introduction: The rapid bone loss in the early postmenopausal oestrogen-dependent phase is followed by a slower rate of bone loss all throughout ageing. In the early phase, bone loss is accompanied by an increase in bone width, which partially preserves bone strength. This periosteal expansion is described by Ahlborg up to age 67 and in cross-sectional studies.

Aims: To determine rates of bone loss in different intervals after menopause; whether the periosteal expansion continues in higher ages; and whether it is associated with the decline in oestrogen levels following menopause.

Methods: 81 women without bone-interfering diseases or medication were followed from menopause and on average 24 years onwards with repeated SPA measurements of the distal forearm. Annual rates of bone loss and bone size were calculated in three intervals: 0-8 years post-menopause, 8-16 years post-menopause and 16-28 years post-menopause. In the first interval, the rates were correlated with mean serum oestrogen levels. A strength index was calculated, taking both bone mass and bone structure into account.

Results: In the three periods, the annual loss in aBMD was 2.0% (1.6, 2.4), 1.0% (0.6, 1.4) and 1.0% (0.7, 1.3), and the annual periosteal expansion was 1.0% (0.8, 1.3), 0.0% (–0.3, 0.3) and 0.0% (–0.2, 0.2). aBMD became significantly lower 5 years after menopause, periosteal diameter significantly wider after 5 years, and the Strength Index only became significantly lower 12 years after menopause. The serum oestrogen levels correlated moderately in the period 0–8 years with the bone loss ($r=0.51$, $p<0.001$) but less with the increase in bone width ($r=-0.22$, $p=0.06$).

Conclusions: Postmenopausal bone loss is highest during the first decade, thereafter it continues at a slow, steady level. Postmenopausal periosteal expansion

in the distal forearm is found only in the first postmenopausal decade and is not strongly associated with oestrogen levels.

Paper IV: Low BMD is an independent predictor of fracture and early menopause of mortality in post-menopausal women – A 34-year prospective study

Introduction: Early menopause has been recognised as a risk factor for both fracture and mortality, and our first paper also described this association. However, it is less investigated whether early menopause is a risk factor in its own right, or whether the risk is mediated by other associated factors. In this paper, we expand our first study by taking a number of potential risk factors for fracture into account, to determine whether the risk associated with early menopause remains after adjustments.

Methods: A set of well-recognised risk factors for fracture were registered at age 48 in 390 north European women who were then followed for 34 years. Fragility fractures and mortality were registered continuously. At age 82, risk ratios for each baseline variable were calculated with Cox's proportional hazard regression analysis.

Aims: To identify risk factors for fragility fractures and mortality in women aged 48, and specifically, to determine whether early menopause is an independent predictor of fragility fracture.

Results: In a multivariate analysis, only baseline BMD remained an independent risk factor for fracture, with a risk ratio of 1.36 (1.15, 1.62) per SD decrease; early menopause reached an almost significant risk ratio. In a multivariate analysis of mortality risk, both early menopause (RR 1.62 [1.09, 2.39]) and smoking (2.16 [1.53, 3.06]) were independent predictors.

Conclusions: Low BMD at age 48 is an independent predictor for fragility fractures. The predictive ability of early menopause is at least partially attributed to other associated risk factors, chiefly low BMD. Early menopause and smoking were found in this study to be independent predictors for mortality.

General discussion

The overall aim of this thesis was to evaluate predictors of bone fragility and fractures in postmenopausal women. Numerous risk factors have been recognised in many previous reports, but there is a shortage of long-term evaluations. Our accumulated knowledge is mainly based on cross-sectional studies, often with fracture data gathered from questionnaires or interviews. However, in order to initiate effective preventive measures, risk factors should be recognised early, long before fracture risk is imminent. All fragility fractures become more common with increasing age, and prospective studies are required to identify risk factors in advance. In women, the onset of menopause heralds a defining period marked by accelerated bone loss with effects that may prevail into high ages. Consequently, the perimenopausal period seems an opportune time for efforts to prevent fragility fractures. It is also uncertain whether the impact of early menopause actually lasts until the ages when fragility fractures rise exponentially in incidence, since some reports indicate a diminishing influence with increasing age [52, 54, 56].

With this perspective, our aim was to increase the level of evidence and verify or reject current beliefs by means of a population-based longitudinal study design where fracture and mortality data were collected from national and hospital registers. The unique length of this study and the prospective design allowed us to estimate predictors of bone fragility and fracture with the starting point in the perimenopausal period and with an endpoint in the ages where fragility fractures have risen exponentially.

In this study, we intended to cover several aspects of bone fragility in women. One focus is early menopause, one of the most important risk factors for bone fragility and fracture in women (**Papers I** and **IV**). Physical activity is one important lifestyle-related health factor that the individual herself can influence, the long-term effects of which are evaluated in **Paper II**. We measure rates of bone loss after menopause and also highlight the changes in bone size, which is an important contributor to bone strength (**Paper III**).

Effects of early menopause

Early menopause is one of the strongest predictors of osteoporosis and fragility fracture in women [52, 82], and has also been associated with high mortality [148]. The widely accepted definition of early menopause refers to menopause before the age of 45. The women in our study were all 48 years old at baseline and were dichotomised according to menopausal status at study start. To be certain to observe the WHO criteria that requires 12 months of amenorrhoea, we counted one year backwards from the age at baseline, which resulted in age 47 as the threshold value. This division into two categories allowed stringent group comparisons, whereas a dichotomisation according to the common level of age 45, or calculations involving the exact age at menopause, would have had to be based on patient recall with risk of bias. In comparison, the average age at menopause in women in the Western world is 51 [24, 60, 62, 99, 100].

The menopausal transition and its implications for the female body are driven by a decline in serum oestrogen levels. This entails comprehensive effects on the female body, among which bone loss is one of the most pronounced. The crucial role of oestrogen in maintaining skeletal health is still poorly understood in terms of mode of action, but bone loss is evidently accelerated during the first postmenopausal decade where approximately 25% of the trabecular bone mass and 15% of the cortical bone mass is lost [44, 124, 126]. Although a steady state prevails thereafter with a continuous slower rate of bone loss throughout ageing, an early onset of menopause confers a higher risk of developing osteoporosis [4, 44, 45, 52, 58, 121, 128]. In addition to bone loss, the peak bone mass acquired during adolescence is a principal determinant for future risk of osteoporosis and fracture [38, 41, 70, 75]. It has been estimated that about half of the variance in bone mass at age 70 is predicted by the peak bone mass [71], which is reached at around age 20 in the hip and vertebrae and in the ages 30–40 in the distal forearm [19, 97, 122, 137, 158]. Low peak bone mass in combination with postmenopausal bone loss is responsible for the higher prevalence of osteoporosis in women compared to men.

Not without reason, the impact of early menopause on osteoporosis and fracture risk has been suggested to attenuate with advancing age [52, 54, 56], given the slower rates of bone loss in higher ages and the accumulation of other risk factors in the elderly woman, e.g. generally impaired health, medication and diseases affecting balance and cognition, poor vision, insufficient nutrition, and decline in muscle mass and mobility. Again, this notion has been based on literature that is predominantly cross-sectional or short-term. Our results in **Paper I** contribute new knowledge by evaluating the long-term impact of a menopause before age 47. In

the women with menopause before age 47, we found an increased risk of osteoporosis, fragility fracture and mortality during a 34-year follow-up period that lasted up to the age where fractures become a problem in magnitude. This suggests that early menopause is a risk factor that should receive active attention regarding both skeletal and general health. Furthermore, our results suggest that also women with a later menopause than the widely accepted cut-off age at 45 are at risk. Lifestyle recommendations should be given already in the immediate postmenopausal period in order to minimise the influence of other potentially harmful factors. Physical activity, proper nutrition, and alcohol and smoking habits are such factors that should be addressed in order to reduce fracture risk. In addition, we suggest BMD be measured in the first decade following menopause, and when appropriate, calcium and vitamin D supplements should be prescribed.

Given the observational design of our study, we cannot explain any causal relationships. While the higher fracture incidence and prevalence of osteoporosis could be attributed to the earlier onset of bone loss, the higher mortality rate in women with earlier menopause seems more unclear. We do not have data on cause of death and can only speculate on a possible association between fracture and death. Surgery- or fracture-related mortality seems unlikely to explain the different mortality rates since only 33 hip fractures occurred in the total cohort, 25 of which in the women with menopause after age 47. Studies have shown a higher overall mortality in women with early menopause [148] and also a protective effect of oestrogen therapy on total mortality [138], but again the effects of oestrogen seem more general than specific, since no clear explanation for these findings has been proposed. On the contrary, the association with breast cancer and thrombo-embolism are well established, and the previously assumed preventive effect on cardiovascular disease was contradicted by controversial data from the WHI [133] and HERS reports [93].

Paper IV is an expansion of **Paper I**, using the same population and outcome variables. The purpose of this study was to identify independent risk factors for fracture, and in particular, whether the predictability of fracture by early menopause seen in **Paper I** was independent or mediated by associated factors. Having included a set of well-known risk factors registered at age 48, our analyses showed that the predictability of fracture by early menopause could be primarily explained by low BMD. Given the close associations between early menopause and low BMD [52], and between low BMD and fracture risk [74, 94], this is not illogical. However, with our observational study design, we cannot prove causal relationships, only associations. The question remains whether the menopausal transition itself and the consequent oestrogen deficiency causes deteriorated health and higher fracture risk, or if unidentified background factors present already at study start could cause both early menopause and higher fracture incidence. In the first case, early menopause would be the causal factor and in the second, a marker

of risk. The higher mortality associated with early menopause seen in **Paper I** was confirmed in **Paper IV**, where we found an independent effect of menopause before age 47. This corroborates previous findings of an association between early menopause and increased risk of overall mortality [148]. Since our study was not designed to evaluate risk factors for mortality, the baseline variables included in the adjustments were originally selected for their association with low BMD and fracture, making the mortality analysis less complete regarding potential confounders at baseline. Moreover, the study does not include data on cause of death or confounders during the follow-up period. In other words, it can only be a matter of speculation whether the cause of death could be related to oestrogen deficiency. Again, the role of menopause as either the cause or the result of generally impaired health remains unclear. Regardless of cause and effect, we suggest that early menopause is an indicator of a premature ageing process and as such a useful predictor of fragility fracture and mortality.

The challenges in estimating the long-term impacts of early menopause are not overcome in this study. The main limitation to our results in **Papers I** and **IV** is the inability to control for confounders during the follow-up period. Since complete data on medication and diseases exist only in the women who attended the follow-up measurements in 2002 and 2006 (56%), we can only speculate on how these potential confounders could affect our results. It could be proposed that mainly women with early menopause would be treated with oestrogen, in which case our conclusions would not be altered. Ideally, every individual should have been followed across her natural menopause in order to decide the exact timing, and with thorough documentation of potential confounders over a long follow-up period. Based on such information a dose-response relationship between age at menopause, BMD levels and fracture risk could be prospected. In this relatively small observational study, about one fourth of the subjects were postmenopausal at baseline and another fourth were excluded at the beginning of the study for various reasons. This meant that age at menopause could not be used for any analysis and instead, we dichotomised the studied population according to menopausal status at baseline.

In sum, the definitive evaluation of how long the impact of early menopause is sustained would require a large prospective study where every individual is followed through her natural menopause and the ensuing decades, and with thorough registration of confounders such as lifestyle parameters, diseases and medication. Despite the acknowledged limitations to our study, we claim to have taken some steps in that direction.

Physical activity and its effect on bone

In **Paper II**, we evaluate the long-term effects of moderate physical activity in 112 postmenopausal women followed with repeated SPA measurements from menopause and 25 years onward. Our results show that annual bone loss was lower in the physically active women, after adjustment for a number of potential confounders including age at menopause and postmenopausal oestrogen levels. BMC did not differ significantly at baseline but was significantly higher in the active group at age 77. Data on activity levels were collected from questionnaires. We suggest that a lifestyle with moderate physical activity such as walking, gardening, cycling or regular exercise is bone-preserving also in a long-term perspective. In addition, a beneficial impact on a number of potential health factors such as body mass index, blood pressure, cardiovascular disease, neuromuscular function, and social and psychological conditions is plausible but beyond the scope of this study.

The effects of physical activity in postmenopausal women have been studied extensively, but the majority of reports concern controlled high-load exercise programmes with duration of at best two years and BMD increments of 1–3% [95, 147, 166]. As regards moderate physical activity, the bone-preserving effects of walking have been analysed in a review by Martyn-St James and Carroll [96] who found that brisk walking over 6–24 months had discrete effects on femoral neck BMD but no effects in lumbar spine BMD. In a large 12-year prospective study by Feskanich et al., four hours of walking per week was associated with a substantial reduction in hip fracture incidence in postmenopausal women [47].

Our study also showed only discrete effects of physical activity, as bone loss was reduced by 0.4% annually. However this was not unexpected, given the moderate level of physical activity and the unloaded measurement site. It is known that long-duration, moderate mechanical load is far less osteogenic than short bouts of dynamic high-intensity loading with intermittent pauses and diversified strain [131, 162]. Furthermore, there are indications that the female skeleton becomes less responsive to mechanical stimuli with declining oestrogen levels [13, 86]. Third, the SPA method measures a non-loaded site, while the impacts of mechanical load are known to be site-specific [85]. Fourth, given the number of factors affecting bone mass during ageing, moderate physical activity could not be expected to be a principal determinant. In order to capture significant differences, we chose a long study period instead of short intervals where the impact of physical activity probably would be obscured by stronger determinants of bone mass. Despite the limitations in study design, we were able to document a significant effect of

physical activity on bone mass. However, we must point out once more that with our observational study design we cannot prove causality; healthy women may be more active and lose less bone mass simply because they are healthy, not because they are more physically active. In other words, the causal link could be between illness and bone loss, and illness and lack of physical activity, and not between lack of physical activity and bone loss. Nevertheless, given the absence of group differences as regards medication and disease at baseline and during follow-up, we argue that the differences in bone loss seen during this 25-year follow-up period do reflect a substantial effect in preserving bone health.

The optimal study design for evaluating effects of physical activity would be a randomised controlled trial, so that levels of mechanical load could be known exactly and allow inferences on causal relationships. However, maintaining such an intervention study with a randomisation of physical activity for 25 years would be virtually impossible.

Bone structure variables are also measured in this study. Periosteal bone width increased as expected in both groups during the study period but no group differences were found. This may be explained by the findings in **Paper III**, where changes in bone size were less pronounced than changes in bone mass. This may suggest that bone size in fact is less responsive to both endogenous and external stimuli than bone mass in elderly women. If so, stronger mechanical load would be required to produce detectable changes in bone width than the moderate general physical activity applied to the subjects in our study.

While changes in bone dimensions are extensively studied in the adolescent skeleton, far less is documented in the elderly. The preference to study young individuals is probably due to their far greater responsiveness to mechanical stimuli, which provides an opportunity to enhance peak bone mass. Polidoulis et al. and Nikander et al. have published meta-analyses where physical exercise in postmenopausal women was evaluated regarding bone geometry [114] and bone strength [105], respectively. No significant effects were found. Again, this indicates that the structural properties of the ageing skeleton are difficult to influence through mechanical load, possibly the more so in postmenopausal women given the reported role of oestrogen as an amplifier of the bone's response [13, 86]. To what extent the micro-architectural properties – the number, integrity and thickness of the trabeculae and cortices – are affected is unknown.

It would be an intriguing prospect to observe the effects of physical activity in different phases throughout ageing. In comparison, pre- and early puberty is a period of intense bone turnover and regarded as a "window of opportunity" for maximising the estimated one third of the peak bone mass that is not genetically predetermined. Since the early postmenopausal period is also marked by an accelerated bone turnover, one could hypothesise that the skeleton could be more

susceptible to mechanical load during this period. However, this would be methodologically challenging given the extremely strong influence of age at menopause and oestrogen levels on bone loss during this period. Exercise programmes with very high osteogenic load would probably be required to produce significant effects. This is one reason for our choosing a long evaluation period.

Estimations of bone strength

Osteoporosis is defined as a chronic systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of the bone tissue, leading to enhanced bone fragility and as a consequence increased fracture risk [8]. However, the operational classification of osteoporosis is entirely based on BMD as measured by DXA, i.e. without regard to the micro-architectural deterioration. Thus the term refers only to the decrease in areal BMD, but in fact the two-dimensional measurement (DXA) of a three-dimensional structure (bone) unintentionally also reflects the bone size – a large bone will yield a higher aBMD value than a small bone even if the true BMD does not differ. Nevertheless, osteoporosis is the established measure for fracture risk estimates, well implemented, easily accessible and proven to correlate well with hip fracture risk especially [74, 94]. Somewhat ironically, it seems that while DXA provides an inexact technical value, the measure obtained is likely to be more useful in practice than true BMD. The explanation is that BMD is only one contributor to bone strength. Although the deterioration of the skeleton's micro-architecture is included in the modern definition of osteoporosis, what is measured is only a material property, areal BMD. The structural component of bone fragility has gained full recognition only in the last decade, and with the growing insights into the geometrical and micro-structural aspects of bone strength, osteoporosis has become a somewhat narrowed description. The general term bone fragility is less exact but may often be preferable as it refers not only to BMD but also to the diverse aspects of bone strength, and the complexity of its estimation.

While BMD is the major determinant of bone strength [20], reduced bone size has also been associated with fracture in both hip [144, 155] and vertebrae [39, 40, 144, 150]. However, the geometrical contributions to bone strength are still estimated mainly through theoretical approaches. Key geometrical parameters such as the cross-sectional moment of inertia and the section modulus can be calculated from the periosteal and medullary diameters which are estimated graphically on SPA and by direct three-dimensional measurement on pQCT. The HSA software developed by Beck et al. [15] and Yoshikawa et al. [173] can be applied to DXA measurements in

order to approximate femoral neck width and cortical area, and from this calculate the section modulus, which represents the bone's ability to resist bending forces. The cross-sectional moment of inertia has been reported to correlate well with distal radius strength in cadavers [9], but neither of these parameters has been associated with fractures. To encompass both structural and material properties in the fragility estimate, a strength index calculated as the product of the section modulus and cortical mineral content has been proposed [68]. The strength index is reported to correlate well with mechanical strength in rat bones [46] but has to our knowledge not been evaluated for fracture predictability in larger epidemiological studies.

The inability of bone measurements to encompass the full fracture risk estimate is due to the fact that the skeletal traits only constitute one leg in the assessment. The second leg is risk of falls, which is mainly associated with poor visual acuity and neuromuscular function, in turn caused by e.g. sensory, balance and cognition disorders, impaired muscular function, hypotension, or medication or diseases affecting cognition and balance [82], all more frequent with advancing age. The third contribution to fracture risk is the exposition to and the energy of trauma, which stresses the need to eliminate extrinsic risk factors in the close environment. Monitoring such hazards is however beyond the scope of this thesis.

In **Paper III**, changes in bone mass and bone geometry were followed with SPA measurements from menopause and 25 years onwards. The study is an extension of an article by Ahlborg et al., who showed that postmenopausal bone loss is accompanied by an increase in bone width up to age 67 [5]. This had previously been indicated by cross-sectional and experimental data, but never demonstrated in prospective studies [14, 21]. In our follow-up study, annual changes were calculated in three intervals in relation to menopause. Our results concur with the common view that bone loss in women is most pronounced in the immediate postmenopausal period which is followed by a lower, gradual decline throughout ageing. This was an expected finding. Since SPA also provides data on bone structure, we evaluated whether the patterns of bone loss were accompanied by corresponding changes in bone size. Would increases in bone width continue to counteract bone loss all throughout ageing? We found that bone width increased during the first eight years following menopause, but not afterwards. Consequently, increases in bone size could partially counteract bone loss and preserve bone strength in the early postmenopausal period but not in the ages when fragility fractures become more common.

The concept of periosteal apposition is an explanation model where bone tissue is assumed to be resorbed at the endocortical surface and deposited at the periosteal surface throughout ageing. Reports that bone resorption mainly occurs on the endocortical surface [11, 51, 112] and that oestrogen inhibits periosteal bone formation

in rats [163] have given rise to this notion. Periosteal apposition could also be conceived as the inversion of bone growth in the adolescent skeleton where oestrogen promotes endocortical but not periosteal bone accrual [18, 140]. Some data have associated increased femoral neck width with decreases in cortical area, as estimated by HSA [165], but periosteal apposition as such has not been described in prospective reports [143]. In our material, the cortices actually grew discretely thicker during the first interval, which is difficult to evaluate given the poorer precision of SPA for cortex estimations. One interpretation is that events on the endocortical and periosteal surfaces are independent, not codependent. This suggests that bone loss could occur by intracortical remodelling throughout the cortex, and not only on the inner aspect. If so, it could not be expected that changes on the endosteal and periosteal surfaces would correlate. The original concept of cortical thinning with increasing bone width has also been challenged by other reports [156], whereas both cross-sectional [14, 21, 127, 136] and longitudinal [5, 164, 165] data support an expansion of the total bone width. Hence, the term periosteal expansion may be more appropriate than periosteal apposition and is the one we use in our paper.

We also monitor serum oestrogen levels in the early postmenopausal period. We found that oestrogen levels were considerably more robustly associated with bone loss than changes in bone width. In our estimations, only 5% of the changes in bone width were attributed to the oestrogen deficiency. We can only speculate on whether this weak association could be explained by assuming that bone size is influenced by other or stronger factors which are not captured by our study design. Nor can we exclude the risk of the calculation being flawed by imprecise measurements of bone size, as discussed below.

With the declining oestrogen levels, one could speculate as to whether an androgen-dominated hormone pattern would prevail. However, results from cross-sectional studies where men were stratified according to age indicate that bone width in men continues to increase well above the age of 80 years [14, 136]. Moreover, the substantial deposition of bone on the outer bone surface in adolescent boys is chiefly androgen-driven. In other words, the unchanged bone width in the seventh and eighth decades seen in our material does not seem consistent with androgen stimulation.

It would have been interesting to evaluate changes in periosteal expansion and the strength index in relation to distal radius fracture incidence. However, the sample size and the number of fractures were too small for such analyses. Furthermore, we noticed that although the study was extended 12 more years the number of additional distal radius fractures was very small, presumably since the rise in this fracture incidence occurs earlier [17, 23].

The intrinsic difficulties in exploring the structural skeletal changes at short intervals must not be underestimated. First, changes in total bone width and

cortical thickness are subtle and hard to detect unless the precision of the equipment is very high – in our paper about 1% annually during the first eight years post-menopause and without measurable changes thereafter. In comparison, the precision with SPA is 1–2% for bone width and up to 8% for cortical thickness, and similar or slightly lower with pQCT. Furthermore, exact patient repositioning is imperative for maintaining high precision. Secondly, all available techniques today are limited in image resolution, and especially in elderly subjects contour detection may be further hampered by ageing-related attrition and other degenerative changes on the bone surface or in soft tissues. Third, all geometrical calculations are based on the assumption that the bone is circular, but neither the femur nor the radius are perfect cylinders. Errors in measuring the diameters are likely to be amplified by this approximation.

Yet another aspect of bone fragility is the changes in the bone's micro-structural properties, which today can only be captured with the high-resolution pQCT technique. Decimation, thinning and disconnection of the trabeculae, and increased porosity of the cortical bone are such parameters influencing bone strength on the micro-architectural level.

Bone densitometry

In **Paper II**, the reported estimate is BMC. Although a simplistic measure, it is in fact the most informative when evaluating bone loss in longitudinal studies. The standard estimate, areal BMD, is affected by changes in bone size, which are present both during growth and in the early postmenopausal period. Consequently, BMC is in fact the most adequate measure for assessing longitudinal changes in the amount of bone mineral, although it cannot answer whether the bone becomes denser or smaller. Prospective studies with repeated measurements also require a technique with high precision, as discussed above. The ability to reproduce the same result in repeated measurements of the same individual is more important than good accuracy (difference between measured value and true value), when only changes are evaluated and not the absolute values. In this study, BMD was measured with single-photon absorptiometry (SPA) which is an outdated technique today. However, the method was the best one available when this study began in 1977 and has the advantage of also measuring the total periosteal width, the medullary width and the cortical thickness, which allows calculation of geometrical parameters of bone strength. SPA is also a method with high enough precision to detect the discrete changes in bone mass and bone size during ageing. Since the 1990s, dual X-ray absorptiometry (DXA) has been the gold standard for bone densitometry and also the basis for the classifications of osteopenia and

osteoporosis. Its moderate radiation doses could make it suitable for a longitudinal study. However, measures of bone size are not provided with DXA, and must be approximated for hip strength analyses. Peripheral quantitative computed tomography (pQCT) provides three-dimensional measures of volumetric, "true" BMD and bone area on trabecular and cortical bone, separately. However, while these pQCT estimates are the most exact that can be assessed today, individually they do not provide comprehensive information. For example, when vBMD became possible to measure, it appeared that it did not differ substantially between adolescent boys and girls [18, 140]; consequently, the differences seen with DXA were apparently attributable to bone size. That is to say, given the complexity of bone strength, exact measures like the volumetric BMD are not always as informative as more general ones. DXA is criticised for producing two-dimensional structures of three-dimensional measures. However, although the measures of areal BMD are falsely elevated in larger bones and inexact in a technical aspect, the unintentional inclusion of the bone size probably means that DXA nevertheless provides the most useful solitary measure of bone strength. Theoretically, the ultimate tool for fracture prediction would be a composite index including both material and structural properties – such as the Strength Index calculated in **Papers II** and **III**. In our reports, too few individuals were included to evaluate the usefulness of the Strength Index for facture prediction, nor has it to our knowledge been tested in larger studies.

Strengths and limitations of the study

The main strength of our study is the unique length of follow-up, providing data collected over 34 years. The studied cohort is population-based and homogeneous as regards age, ethnicity and environmental expositions, since all were recruited from the population records of the city of Malmö. Furthermore, the women were born in the same year and followed from the same chronological baseline. In the women who were premenopausal at baseline (**Papers II** and **III**), timing of menopause was determined to the exact day. When the women who were post- or perimenopausal at study start (**Papers I** and **IV**) were included, the entire cohort was dichotomised according to menopausal status at baseline and using the WHO criteria. In this way we avoided the recall bias present when women are asked to remember their age at menopause in retrospect. Fracture data collection was also obtained continuously through a well validated system, instead of through interviews and patient recall. The repeated bone measurements were conducted using a method with high precision, which increases the reliability of the measured results. In **Papers II** and **III**, data on lifestyle, medication and general health were collected repeatedly.

The main limitation to our study is the lack of data on the aforementioned potential confounders. The results in **Papers I** and **IV** are afflicted with uncertainties because of the inability to control for such factors. Bone measurements were undertaken with SPA, which has lower accuracy than DXA and measures an unloaded cortical site, whereas DXA measures the axial skeleton. The observational study design is also a limitation, since conclusions on causal relationships are not possible.

Summary and future perspectives

Given the projected increase in the proportion of elderly in the population, the total number of fragility fractures is likely to increase in the future. Consequently, the incentive for research to identify risk factors and prevent fractures will continue to grow stronger, from both the individuals' and society's point of view.

In this 34-year prospective observational study, we have evaluated predictors of bone fragility and fractures in postmenopausal women, who are the most exposed to fracture risk. We found that menopause before age 47 is associated with increased risk of osteoporosis, fragility fracture and mortality also in a long-term perspective. After adjustments for confounders present at baseline, the predictability of fracture risk by early menopause seems mediated by low BMD. Menopause before age 47 was independently associated with mortality. We found that bone loss is highest during the first postmenopausal decade and thereafter continues at constant, slower rates. Periosteal expansion occurred only in the first menopausal decade, and not subsequently. Moderate physical activity during 25 years after menopause reduced bone loss but did not affect bone structure.

We suggest that women with menopause before age 47 should be given lifestyle recommendations in the immediate postmenopausal period in order to preserve skeletal and general health. Physical activity can be recommended to postmenopausal women to minimise bone loss.

Our study cannot prove causal relationships and we look forward to future studies in this field. Evaluating long-term effects of risk factors for bone fragility and fracture requires large prospective studies with adequate determination of age at menopause, thorough registration of numerous potential confounders at baseline and during the follow-up period, and continuous collection of fracture and mortality data including cause of death. This would be a laborious task but contribute enormously to our knowledge. Physical activity should ideally be evaluated with long-term randomised intervention programmes of osteogenic high-impact exercises, with three-dimensional densitometry and appropriate

adjustment for confounders. Long-term changes in bone density and bone structure should ideally be measured continuously with three-dimensional techniques that could evaluate material, geometrical and micro-architectural properties. The development of new bone densitometry methods is in steady progress, and perhaps the modest recent contribution to our arsenal, the high-resolution pQCT, can take us further down the road to wisdom.

Conclusions

Women with menopause before age 47, compared to women with later menopause, had a significantly increased risk of having developed osteoporosis at age 82, and a higher risk of mortality and of sustaining a fragility fracture during the following 34 years.

In our model, low BMD at age 48 was the only independent significant risk factor for sustaining a fragility fracture during the following 34 years. Menopause before age 47 was an independent predictor of mortality but not of fracture, suggesting that its predictability of fracture is mediated by other factors, mainly low BMD.

Women who were moderately physically active had a lower bone loss during the 25-year follow-up period than women who were inactive, and had a higher bone mass at 77.

Moderate physical activity does not seem to affect bone structure in postmenopausal women.

Bone loss was most rapid during the first eight years after menopause when there was an annual loss of 2%. Thereafter it continued at a rate of 1% annually in higher ages.

Periosteal expansion was found during the first eight years after menopause when bone size increased by 1% per year, partially counteracting the decreased bone strength caused by bone loss. Subsequently there was no further significant increase in bone size.

Mean estradiol levels correlated moderately with the mean annual bone loss but less with the changes in bone size in the early postmenopausal period. The perimenopausal changes in estradiol levels did not correlate with bone loss or changes in bone size.

Summary in Swedish – Populärvetenskaplig sammanfattning på svenska

Benskörhet (osteoporos) är ett tillstånd som definieras av låg benmassa och ökar med stigande ålder. Osteoporos är idag en av de stora folksjukdomarna och man räknar med att en tiondel av alla män och en fjärdedel av alla kvinnor i Sverige drabbas. Tillståndet är i sig symptomfritt men ökar risken att råka ut för ett benbrott (fraktur) redan vid lättare fall. Frakturer i handled, fotled, höftled, bäcken och ryggkotor är typiskt förknippade med osteoporos och uppgår i antal till ca 70 000 per år i Sverige. Förutom det personliga lidandet är också den ekonomiska kostnaden kännbar för samhället. Mest drabbande för både patient och samhälle är höftfrakturen, som har beräknats uppta 3 % av sjukvårdens resurser. Sammantaget gör detta att osteoporos är ett högaktivt forskningsområde, då incitamenten att finna metoder att bromsa och mildra utvecklingen av benskörhet är starka.

En mängd riskfaktorer för benskörhet och benbrott har identifierats. Rökning, viktnedgång, en stillasittande livsföring och vissa läkemedel såsom kortison är några sådana riskfaktorer. En stark orsak till att benskörhet är så mycket vanligare hos kvinnor än hos män är de sänkta hormonnivåerna i kvinnokroppen som klimakteriet medför. Klimakteriet (menopausen) markerar att kvinnans äggstockar slutar att producera östrogen, vilket utöver upphörda menstruationer innebär stora genomgripande förändringar för kvinnokroppen. Ett av de organ som påverkas mest av de sjunkande östrogenhalterna är skelettet. Det är väl belagt i vetenskapliga studier att en tidig menopaus innebär ökad risk att drabbas av benskörhet och frakturer. Eftersom antalet faktorer som påverkar risken för benskörhet och frakturer kan antas öka med stigande ålder, är det dock ovisst hur länge effekten av en tidig menopaus varar. Detta har inte kunnat besvaras i de studier som gjorts på ämnet, då de antingen haft för kort uppföljningstid eller varit så kallade tvärsnittsstudier, d.v.s. analyserat vid endast ett tillfälle och inte över tid.

Studien som ligger till grund för den här avhandlingen startade 1977 som ett samarbete mellan kvinnokliniken och ortopediska kliniken på dåvarande Malmö Allmänna Sjukhus, MAS. 390 kvinnor som samtliga var födda 1929 fick vid

studiestart genomgå benmätning i handleden och lämna uppgifter om hälsa och livsstil. De kvinnor som ännu inte genomgått klimakteriet erbjöds att delta i en prospektiv studie (framåtblickande över tid) för att följas genom sin menopaus med upprepade hormon- och benmätningar. Kvinnornas menopausålder kunde därmed bestämmas exakt, enligt de kriterier som satts upp av FN:s världshälsoorgan WHO. Initialt utfördes benmätningar vartannat år vilket sedermera glesades ut. Den sista mätningen genomfördes vid 77 års ålder och vid 82 års ålder registrerades frakturer och dödstal genom databassökningar.

Benmätningarna utfördes med metoden SPA (single-photon absorptiometry) som idag är en föråldrad teknik men som har fördelen att utöver benets täthet kunna mäta också benets och märghålans vidd. Skelettets hållfasthet mot fraktur beror nämligen inte bara på benets täthet utan också på dess storlek och inre mikroskopiska struktur.

Syftet med denna avhandling var att studera och identifiera riskfaktorer för osteoporos och frakturer hos kvinnor som passerat menopaus. Uppföljningstiden på 34 år gör studien unik i sitt slag, liksom att vi kunnat bestämma datum för menopaus exakt och använda detta som utgångspunkt för våra longitudinella mätningar. Detta innebar att vi kunnat utvärdera riskfaktorer och förändringar i skelettet specifikt i förhållande till menopausen.

I *det första delarbetet* i avhandlingen delades de studerade 390 kvinnorna in i två grupper: en grupp bestående av de 61 kvinnor som genomgick menopaus före 47 års ålder, och en grupp på 329 kvinnor med menopaus vid 47 års ålder eller senare. Vi jämförde sedan bentätheten vid 77 års ålder, och frakturer och dödstal fram till 82 års ålder. Den statistiska analysen visade att förekomsten av både osteoporos, frakturer och förtidig död var signifikant högre i gruppen med tidig menopaus. Detta är den första studie som visat att tidig menopaus medför en ökad risk så länge som fram till 82 års ålder. Kvinnor med tidig menopaus bör därför identifieras tidigt för att kunna ges livsstilsrekommendationer och vid behov genomgå bentäthetsmätningar.

I *det andra delarbetet* studerades 112 kvinnor avseende betydelsen av fysisk aktivitet. Kvinnorna fick i enkäter redovisa hur mycket regelbunden vardaglig motion i form av promenader, trädgårdsarbete, schemalagd träning etc. de haft under hela uppföljningstiden. De delades sedan in i två grupper enligt ett gränsvärde på 30 minuters fysisk aktivitet per dag, vilket är den miniminivå som använts i amerikanska folkhälsorekommendationer. Den årliga benförlusten beräknades sedan mellan menopaus och 77 års ålder, och som väntat minskade bentätheten i bägge grupper. Minskningen var dock signifikant mindre hos de 91 kvinnor som var fysiskt aktiva, och bentätheten vid 77 års ålder var också högre än i den inaktiva gruppen. Dessa skillnader kvarstod efter att man justerat för andra faktorer såsom ålder vid menopaus, östrogennivåer mm. Resultaten tyder på att en

livsstil med regelbunden måttlig fysisk aktivitet kan rekommenderas som en metod att bibehålla benmassa.

I *det tredje delarbetet* mättes förändringar i bentäthet och benstorlek i olika tidsperioder efter menopaus. Det är känt sedan tidigare att menopausen följs av en fas med snabb östrogen-beroende förlust i bentäthet, följd av en konstant, lägre grad av åldersrelaterad benförlust under återstoden av livet. Parallellt med benförlusten sker en ökning av benvidden som i viss mån bevarar benets hållfasthet och styrka. I den här studien studerades 81 kvinnor från menopaus och 24 år framåt. Vi fann att bentätheten minskade med 2 % under de första åtta åren efter menopaus, och därefter med 1 % årligen fram till studiens slut med 76 års ålder. Vidare såg vi att benstorleken ökade med 1 % under de första åtta åren, varefter det inte skedde någon ytterligare ökning av benstorleken. Förlusten i bentäthet korrelerade i viss mån med östrogennivåerna efter menopaus, till skillnad från förändringen i benvidd. Resultaten stödjer hypotesen att ökning av benvidden kan vara en mekanism som avseende benstyrkan kompenserar förlusten i bentäthet under det första decenniet efter menopaus, men inte därefter.

Det fjärde delarbetet är en fördjupning av det första delarbetet. Utöver tidig menopaus inkluderade vi nu ett flertal kända riskfaktorer för osteoporos och fraktur som noterades vid 48 års ålder, och utvärderade vilka som kunde förutspå risken för fraktur eller död under uppföljningstiden. När vi tog med dessa registrerade faktorer i beräkningen fann vi att det endast var låg bentäthet vid 48 års ålder som ensamt kunde förutspå fraktur. Menopaus före 47 års ålder medförde inte en ökad risk oberoende av andra faktorer. Däremot medförde både rökning och menopaus före 47 år oberoende av andra faktorer en signifikant ökad risk att dö under uppföljningstiden. Resultaten kan tolkas på olika sätt. Menopaus före 47 år kan vara associerat med fraktur via andra faktorer som är förknippade med tidig menopaus. En annan förklaring kan vara att det finns en bakomliggande, oidentifierad grundorsak till både tidig menopaus och ökad frakturrisk. På så sätt blir menopaus före 47 år en indikator och inte en grundorsak. Samma resonemang gäller för risken att dö under uppföljningstiden. Menopaus före 47 år kan vara orsaken till ökad dödlighet, eller vara följd av bakomliggande omständigheter som orsakar både tidig menopaus och ökad dödlighet.

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Appendix

DAGENS Medicin

VETENSKAP

Publicerad 2012-04-27 *Vetenskap: Kvinnohälsa*

Kvinnor med tidig menopaus levde kortare

Att få sin sista mens före 47 års ålder är kopplat till ökad frakturrisk och sämre överlevnad. Det visar en svensk studie i tidskriften British Journal of Obstetrics and Gynaecology, BJOG

Tidigare tvärsnittsstudier och andra framåtblickande studier har visat att det finns en koppling mellan tidig menopaus och benskörhet och frakturrisk i ett kortare perspektiv. Vår studie är dock den hittills klart längsta inom området, säger Ola Svejme, doktorand och ST-läkare vid ortopedkliniken på Skåne universitetssjukhus i Malmö.

Hans och hans kollegors studie omfattar 390 kvinnor som föddes i Malmö 1929. När kvinnorna var 48 år fick de göra en första bentäthetsmätning, som upprepades trettio år senare.

Totalt hade 61 deltagare en tidig menopaus, med sin sista mens före 47 års ålder. Dessa hade en nästan fördubblad risk att vara bensköra vid den andra mätningen, jämfört med majoriteten med sen menopaus.

En tidig menopaus var också kopplat till 70 procents ökad risk att ha drabbats av fraktur och 60 procent ökad risk för att ha avlidit under 34 års uppföljning, jämfört med kontrollgruppen.

Totalt sett hade 52 respektive 35 procent av kvinnorna avlidit i grupperna.

Ola Svejme betonar att undersökningen är en observationsstudie och därför inte kan visa några orsakssamband.

– Men det kan vara så att bortfallet av östrogen efter menopaus har så omfattande påverkan på kroppen att det kan förklara de ökade riskerna för fraktur och tidig död. Det kan också vara så att andra grundtillstånd ligger bakom både den tidiga menopausen och den ökade riskerna vi ser. Här behövs mer forskning, säger Ola Svejme.

En tidig menopaus bör dock betraktas som en riskfaktor, anser han.

– Det kan vara motiverat med tidigare mätningar av bentäthet i denna grupp och i förekommande fall behandling med kalk och d-vitamin. Även livsstilsinsatser, som ökad mängd fysisk aktivitet kan stärka skelettet, säger Ola Svejme.

Tidig menopaus, före 47 års ålder, förekommer hos runt 15 procent av kvinnorna i Sverige

Article of the Month - September Issue 2012

Title: Physical activity reduces bone loss in the distal forearm in post-menopausal women - a 25-year prospective study.

Authors: Ola Svejme, Henrik Ahlborg, Magnus Karlsson.

Published in: Scandinavian journal of medicine & science in sports. Epub 2012 Jul 30.

Editorial Comment

Falls and fractures are common among elderly individuals. Fractures lead to pain, suffering, and immobility, and those caused by bone loss will be an increasing medical problem in our aging population. Moderate physical activity may be one approach to reducing bone loss, increasing bone strength, and lowering the number of fractures. This possibility was evaluated by Svejme et al., as described in the article “Physical activity reduces bone loss in the distal forearm in post-menopausal women - a 25-year

prospective study" published in the Scandinavian journal of medicine & science in sports.

This study is unique because of its extensive follow-up time and prospective design with multiple absorptiometry measurements. Another novel aspect is that time of menopause was used as baseline and was defined according to the WHO criteria and laboratory tests. That made it possible to interpret the results in relation to the hormonal depletion, although the authors clearly emphasized that their findings cannot answer the question of a causal association between physical activity and less bone loss.

Changes in bone mass and bone structure were measured by repeated single-photon absorptiometry of the distal forearm in 91 moderately active and 21 inactive women, from the time of menopause through the next 25 years. For dichotomization, Svejme and colleagues used a threshold value of 30 minutes of general, moderate physical activity per day, representing the minimum amount of daily physical activity specified in public health recommendations. The mean annual loss of bone mineral content was greater in the inactive women, as was the mean decline in a strength index based on bone mass and bone structure.

The results of this study are particularly valuable considering the long follow-up time of 25 years after menopause, as well as the fact that it is now difficult to reproduce this type of research, because many women today take medications to prevent osteoporosis. Accordingly, the data obtained are highly beneficial and strongly indicate that physical activity (30 min/day) should also be recommended for postmenopausal women.

“Gloria Orthopaediae in Eternum”

“Heja di blåe!”

Paper I

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Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women

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Objective A prospective evaluation of the long-term effects of early menopause on mortality, risk of fragility fracture and osteoporosis.

Design Prospective population-based observational study.

Setting Malmö, Sweden.

Population A total of 390 white north European women aged 48 years at the start of the study.

Methods At baseline, bone mineral density (BMD) was measured by single-photon absorptiometry (SPA) in the distal forearm and menopausal status was noted. Menopause was determined according to the World Health Organization criterion of a minimum of 12 months of continuous amenorrhoea. Women were divided into early menopause (occurring before age 47 years) and late menopause (occurring at age 47 years or later). At age 77, forearm BMD was re-measured by SPA and proximal femur and lumbar spine BMD were measured by dual-energy X-ray absorptiometry (DXA). The prevalence of osteoporosis was determined using the DXA data. Mortality rate and the incidence of fractures were registered up until age 82. Data are presented as means with 95% confidence intervals (95% CI).

Main outcome measures Incidence of fragility fractures, mortality, prevalence of osteoporosis at age 77.

Results Women with early menopause had a risk ratio of 1.83 (95% CI 1.22–2.74) for osteoporosis at age 77, a risk ratio of 1.68 (95% CI 1.05–2.57) for fragility fracture and a mortality risk of 1.59 (95% CI 1.04–2.36).

Conclusions Menopause before age 47 is associated with increased mortality risk and increased risk of sustaining fragility fractures and of osteoporosis at age 77.

Keywords Bone mass, fractures, menopause, mortality, osteoporosis.

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Introduction

Published literature suggests that an early menopause can predict osteoporosis and its clinical manifestation, fragility fractures.[1–6] However, opinions differ, because some long-term studies have concluded that age at menopause does not influence bone mineral density (BMD) after the age of 70 years.[7–9] Furthermore, most studies on the subject have been cross-sectional rather than population-based cohorts and have used retrospective definitions of menopause age obtained through interviews or questionnaires—a study design subject to recall bias. In addition, most studies have evaluated outcome in the first decade following the menopause,[1] whereas the risk of fracture only starts to increase exponentially several decades after the menopause,[10] and the mean age for a female hip fracture in Sweden is currently 82 years.[10]

An ideal study to estimate the long-term impact of menopause on the prevalence of osteoporosis and fracture risk should follow a homogeneous population of women from the perimenopausal period through several decades of life until the participants reach the age when osteoporosis and fragility fractures become a common problem.

The Malmö Perimenopausal Study is one such population-based prospective observational study where women have been followed from age 48 years onwards. The 16-year follow-up data have been presented,[8] and the follow up in the current report is extended to a total of 29 years for the BMD measurements and 34 years for the fracture and mortality evaluations.

In this study we hypothesise that an early menopause is a risk factor for osteoporosis, mortality and fragility fractures in a long-term prospective study.

Methods

In 1977, 390 women aged 48 years were recruited to this prospective observational study, originally with the aim of evaluating changes in BMD and endocrine parameters in the perimenopausal and postmenopausal periods.[11,12] All white north European female residents of the city of Malmö, Sweden who were born during the latter half of 1929 were selected from the city population records.[11] At age 77 years, all eligible participants were re-invited for BMD measurements. At this point, 298 women were still alive and 92 had died. There was no information available on cause of death. One hundred of the 298 women still alive had relocated or declined further participation because of disease or for personal reasons, leaving 198 women to attend the follow-up measurement 29 years after baseline. Lifestyle parameters, medical conditions, medications used and gynaecological history were noted at baseline and at age 77. At baseline, the women were asked specifically whether they were still menstruating or not. We then used the WHO definition that requires 12 months of continuous amenorrhoea to define menopause.[13] Based on this information we were able to divide the women into two categories; an early menopause group consisting of women in whom the menopause occurred before age 47 and a late menopause group including women in whom the menopause occurred at age 47 or later. The mean age at menopause in the early menopause group was 42 years, compared with the average age at menopause of 51 years in the western world.[14–18]

Bone mineral density was measured by single-photon absorptiometry (SPA), which gauges BMD at one mainly trabecular, and one dominantly cortical site of the forearm, as described by Nauclér et al.[19] The SPA measurements were performed both at age 48 and at age 77. The prospective evaluation of bone mass therefore spanned 29 years. The same apparatus and the same anatomical site were used and all data from both measurements were analysed by the same technician. Measurements of a standardised phantom every second week all through the study period did not reveal any long-term drift of the densitometer. At age 77, BMD (g/cm^2) was also measured by dual energy X-ray absorptiometry (DXA; GE Lunar Corp., Madison, WI, USA; Lunar Prodigy®) in the hip and lumbar spine. The precision of the BMD measurements ranged from 0.5 to 3%, depending on application.[20] Osteoporosis was defined according to the WHO definition, i.e. −2.5 standard deviation (SD) from the reference value in young adults.[21] In absolute numbers, these cutoff values were at 0.706 g/cm^2 for hip BMD and 0.907 g/cm^2 for lumbar spine BMD. Height and weight were measured by standard equipment at both 48 and 77 years.

We identified fractures that occurred in these 390 women from repeated searches of hospital registrations and digitised databases, from age 48 years until death, relocation or until the endpoint date 30 September 2011 (age 82 years). The fracture registration period therefore spanned 34 years, and as the incident fractures were objectively registered through their hospital attendances, all women contributed data, even those who declined follow-up BMD measurements. Fragility fractures were defined as low-energy fractures (a fall on the same level) of the wrist, proximal humerus, spine, hip, pelvis, tibia condyle and ankle. Nonclinical asymptomatic vertebral fractures and nonosteoporotic high-energy fractures were not included in our calculations. This fracture ascertainment method has been thoroughly evaluated and used in numerous epidemiological fracture studies.[22–24] Everyone in the Malmö region attends the same trauma unit because there is only one emergency department in the city, and all radiographic examinations were routinely examined by two radiologists and then registered and indexed. Radiographs and reports have been kept on file for each person since the beginning of the twentieth century. Additionally, residents of Malmö who sustain fractures in other geographical regions are referred to the Orthopaedic Department of Skåne University Hospital in Malmö for follow up, at which the fracture is classified in the records as low or high energy. Fewer than 3% of all fracture patients in the city visit a private physician. Most of these injuries would have been minor fractures not necessitating treatment, such as non-dislocated digit fractures.[22–24] Classified fractures were verified by the radiologists' original reports. Nine participants who had relocated to other regions in Sweden were telephoned and asked whether they had sustained any fracture. If fractures were reported, they were verified through case reports acquired from the respective hospital. In 11 women, fracture records could not be obtained because these women had either died after having relocated or could not be located. In these cases, we used the relocation date as the endpoint data in the risk calculations. Mortality data during the 34-year follow-up period was provided from the national population registers. A flow-chart following the 390 women until their last BMD measurement and the end of fracture and mortality registration is shown in Figure 1.

Approval was obtained from the Ethics Committee of Lund University and the study was conducted in accordance with the norms of the Helsinki Declaration of 2001. Written informed consent for collection of data was obtained from each individual. The technical equipment was validated by the Swedish Radiation Protection Inspectorate and by the hospital's own radiation protection

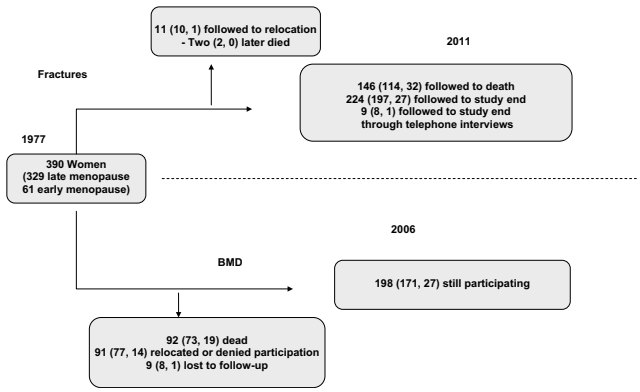

Figure 1. Flow-chart of the participants, 390 at study start, until the last BMD measurement in 2006 and the fracture registration until 30 September 2011. At the last BMD measurement in 2006, 198 of the 298 women still alive (66%) attended. Fracture registration from study start until 30 September 2011 was complete in 379 women (97%), in 370 cases through the hospital archives and the regional databases and in nine women through telephone interviews. Within brackets are numbers for the late menopause group and the early menopause group, respectively.

committee. The Swedish Data Inspection Board approved both the data collection and the database. Statistical processing was carried out using STATISTICA software version 7.1 (StatSoft, Milton Keynes, UK). Data are shown as means with 95% confidence intervals (95% CI). Group comparisons between women with early and late menopause were performed using chi-square tests, log-rank tests, risk ratio and rate ratio calculations and the Student's *t* test between means. Fracture incidence and mortality rate in the two cohorts following baseline were calculated taking person-years into account and are presented with Kaplan–Meier survival curves.

Results

At age 48, no group differences were seen with regards to age at menarche or anthropometrics, whereas distal forearm BMD was 0.43 SD (95% CI 0.14–0.72) lower in the early menopause group (Table 1).

At age 77, 56% (15/27) of women with early menopause had osteoporosis, in comparison with 30% (52/171) of women with late menopause ($P = 0.01$), resulting in a risk ratio of 1.83 (95% 1.22–2.74) (Table 2).

During the 34-year fracture follow-up period, 33% (128/390) of the women had sustained at least one fragility fracture (Table 2). In the early menopause group, the fracture incidence per 10^3 person-years was 19.45 compared with 11.60 in the late menopause group, giving a risk ratio of 1.68 (95% CI 1.05–2.57) for sustaining a fragility fracture (Table 2, Figure 2).

Mortality rate was 52.4% (32/61) in the early menopause group and 35.2% (116/329) in the late menopause group, giving a relative mortality risk of 1.59 (95% 1.04–2.36) (Table 2, Figure 3).

Discussion

The results of this population-based prospective observational study demonstrate that an early menopause is a significant risk factor for osteoporosis, fragility fracture and mortality. There is an abundance of surveys associating early menopause with osteoporosis and risk of fracture. However, the majority of those studies have been cross-sectional and ascertained menopausal age retrospectively.[1,4–6,25–27] To our knowledge, this is the first report with a prospective study design and a follow-up period of more than three decades. In addition, we not only included osteoporosis as the surrogate endpoint marker, but also ascertained the clinically relevant endpoints; fracture and mortality.

Previous reports have generally been robust in establishing the association of early menopause with the presence of low BMD and fragility fractures during the first postmenopausal decade, whereas this relationship seems to disappear with increasing age.[1] Our study was designed to evaluate the influence of age at menopause on the risk of osteoporosis as well as fracture and mortality.[1,4,5,26,27] Our results corroborate most of the previously published data. In one review in 2007 that included predominantly cross-sectional studies and prospective short-term studies, Gallagher[1] concluded that most reports support the association of early menopause with the risk of fracture and future osteoporosis. Gardsell et al.[2] followed 733 women for 11 years and were able to establish a relationship between early menopause and increased fracture incidence but only up to age

Table 1. Characteristics of the 390 women of the original cohort when evaluated at study start at age 48 years

Variable	Early menopause (n = 61)	Late menopause (n = 329)	P value*
Age (years)	48.3 (48.3–48.3)	48.3 (48.3–48.3)	–
Menarche (years)	13.9 (13.4–14.3)	14.0 (13.9–14.2)	0.45
Height (cm)	163.8 (162.3–165.3)	164.1 (163.5–164.6)	0.78
Weight (kg)	63.2 (60.8–65.8)	63.5 (62.4–64.6)	0.88
Forearm BMD (g/cm^2)	0.52 (0.50–0.54)	0.55 (0.54–0.55)	0.002
Menopausal age (years)	42.1 (40.8–43.4)	N/A	
History of breastfeeding			
Yes	42 (69%)	260 (79%)	0.05
No	18 (30%)	60 (18%)	
Missing data	1 (1%)	9 (3%)	
Children			
0	15 (25%)	37 (11%)	0.31
1–3	40 (65%)	271 (82%)	
>3	6 (10%)	20 (6%)	
Missing data	0	1	
Current physical activity			
High	12 (20%)	96 (29%)	0.13
Low	49 (80%)	233 (71%)	
Current smoking			
Yes	33 (54%)	153 (46%)	0.14
No	22 (36%)	158 (48%)	
Missing data	6 (10%)	18 (6%)	
History of oral contraceptives			
Yes	10 (16%)	86 (26%)	0.10
No	51 (84%)	243 (74%)	
Current calcium intake			
<400 mg/day	14 (23%)	56 (17%)	0.27
≥400 mg/day	47 (77%)	273 (83%)	

Data presented as mean with 95% CI or as numbers with proportion (%).
BMD was measured by SPA at the distal radius. Chi-square tests and Student's t test were used for P value calculation.
*P value for early menopause versus late menopause.

70 years. Johansson and Mellstrom,[25] in a cross-sectional retrospective study of 7549 women from six different birth cohorts, reported that fracture risk in each cohort was obviously associated with menopause occurring before the age of 49. However, in contrast to the present study, both menopause age and fracture incidence were estimated retrospectively through questionnaires and high-energy-related fractures were also included in the calculations. In a study of 555 Californian women aged 60–89 years and with 23–34 years of postmenopausal period, Kritz-Silverstein and Barrett-Connor[6] found lower BMD in women with menopause before age 48. However, no fracture data were included in this report. Furthermore, few have evaluated whether an early menopause influences the level of BMD or fracture incidence decades after the menopause, and the sparse published data suggest a fading impact of early menopause with increasing age.[1] This may be caused by the fact that the rapid estrogen-associated bone loss in the first postmenopausal decade is replaced by a slower age-related loss in BMD and that an increasing number of other risk factors for low BMD and fracture appear in older woman, obscuring the effect of early menopause.[2,7–9]

The conflicting results in the literature may be explained by the use of different cutoff ages for early menopause. With a lowering of the threshold when defining early menopause, it seems as if the association between age at menopause and risk of osteoporosis and fragility fracture is strengthened.[4–6,26,27] A cross-sectional study including 1050 Argentinean women aged 50–88 years suggested that women who reached the menopause before age 45 had lower BMD than women with later menopause, and half of the 49 women who had sustained a hip fracture were in the early menopause group.[27] A French cross-sectional study of 1667 women reported that women in whom the menopause had occurred before age 40, two decades later had lower BMD than women with higher menopause age.[5] Van der Voort et al.,[4] in one cross-sectional evaluation involving 4725 women, concluded that a menopause before the age of 45 was associated with a higher fracture risk above age 70 but lower BMD only up to the age of 65.

Table 2. Characteristics of the 198 women who were still participating in the measurements at study end

Variable	Early menopause group	Late menopause group	*P* value*
Age (years)	76.8 (76.6–77.1)	76.5 (76.4–76.6)	–
Age at menopause (years)	42.1 (40.8–43.4)	51.0 (50.7–51.4)	<0.001
Height (cm)	160.3 (157.9–162.6)	160.7 (159.9–161.7)	0.66
Weight (kg)	67.5 (62.9–72.1)	67.3 (65.5–69.2)	0.96
BMD forearm (g/cm^2)	0.36 (0.32–0.40)	0.37 (0.36–0.38)	0.58
BMD hip (g/cm^2)	0.79 (0.74–0.84)	0.83 (0.80–0.85)	0.23
BMD lumbar (g/cm^2)	0.98 (0.90–1.07)	1.04 (1.01–1.07)	0.19
History of hormone replacement therapy			
Yes	6 (22%)	16 (10%)	0.05
No	21 (78%)	155 (90%)	
History of bisphosphonates			
Yes	2 (7%)	20 (12%)	0.51
No	25 (93%)	151 (88%)	
History of oral corticosteroids			
Yes	1 (4%)	10 (6%)	0.65
No	26 (96%)	161 (94%)	
Deceased			
Yes	32 (52%)	116 (35%)	0.01
No	29 (48%)	213 (65%)	
Person-years	1719	10 007	
Osteoporosis			
Yes	15 (56%)	52 (30%)	0.01
No	12 (44%)	119 (70%)	
Fragility fractures			
Yes	27 (44%)	101 (31%)	0.04
No	34 (56%)	228 (69%)	
Person-years	1389	8719	
Distal radius fractures			
Yes	11 (18%)	53 (16%)	0.71
No	50 (82%)	276 (84%)	
Person-years	1583	9074	

Data are presented as mean with 95% CI or as numbers with proportion (%). BMD was measured by SPA at the distal radius (n = 176) and by DXA in total hip (n = 194) and lumbar spine (n = 197).
Osteoporosis was classified according to the WHO classification[14] as a BMD *T*-score below 2.5 SD measured by DXA.
Fracture data are gathered from the 379 women available for fracture follow up.
Chi-square tests and Student's *t* test were used for *P* value calculation.
**P* value for early menopause versus late menopause.

Finally, the Rotterdam study[26] inferred a relative risk of incident vertebral fractures of 2.7 in women with menopause before age 46 but a relative risk of only 1.3 in women with menopause between age 46 and 50, in comparison with women who had menopause after age 50. In summary, these reports indicate a dose–response relationship between age at menopause and fracture risk, i.e. the earlier the onset, the more sustained the impact.

There are also studies that oppose an association between early menopause and osteoporosis and increased fracture risk. In a cross-sectional report by Gerdhem and Obrant,[7] 1044 75-year-old women were asked about their age at menopause, which did not correlate with BMD at age 75. The same conclusion was drawn by Francucci et al.,[9] who studied 782 women and reported that menopause before age 44 was associated with lower BMD up to age 55 but not beyond, indicating a fading effect of age at menopause on BMD with advancing age. In contrast to these cross-sectional evaluations, our prospective data illustrate that the effect of early menopause results in a significantly higher prevalence of osteoporosis at age 77 in women with early menopause as well as a higher incidence of fractures. The discrepancy in study conclusions could be the result of different study designs, the different strategies of estimating age at menopause and the different definitions of early and late menopause.

The reason for the higher fracture risk among women with early menopause can only be speculated upon. One of

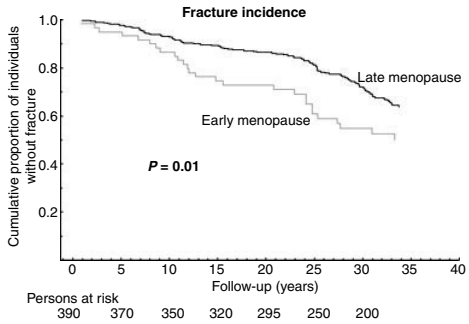

Figure 2. Kaplan–Meier curve presenting fracture incidence in the two groups during the follow-up period. Remaining individuals at risk are provided along the time axis. *P* value is calculated through log-rank test. Rate ratio for fragility fracture incidence taking person years into account was 1.68 (95% CI 1.05–2.67).

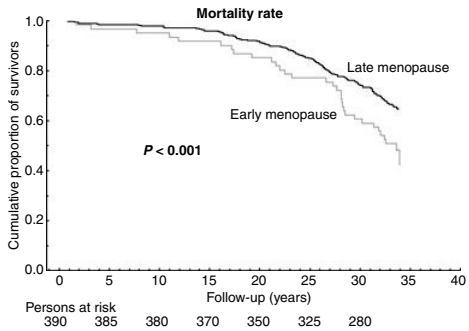

Figure 3. Kaplan–Meier curve presenting mortality rate in the two groups during the follow-up period. Remaining individuals at risk are provided along the time axis. *P* value is calculated through log-rank test. Relative mortality risk taking person-years into account was 1.59 (95% CI 1.04–2.36).

the most recognised risk factors for fractures is low BMD. It is well established in prospective epidemiological studies that a decrease of 1.0 SD increases fracture risk by 50%.[28] The higher fracture risk in women with early menopause in the current study is probably to some extent mediated by a lower BMD, as the early menopause cohort had a significantly higher risk of osteoporosis at age 77 and already, at age 48 years, had on average a 0.4 SD lower BMD than those with late menopause. However, the lower BMD level does not seem to be entirely able to explain the increased fracture risk. We must therefore speculate as to whether factors beyond bone mass, such as inferior muscle strength or inferior neuromuscular function, ought to be found in women with early menopause.

Any possible relationship between the high mortality risk in women with early menopause can likewise only be a matter of speculation. It has been suggested that the higher mortality rate in women with an early menopause is mediated through higher co-morbidity[29] and effects dependent on the hormonal transition associated with menopause.[29] A higher fracture incidence in women with an early menopause could be associated with increased fracture-related mortality,[30–32] but the differences in mortality risk could also be related to group differences in general diseases, medication, nutritional intake, smoking and alcohol habits, level of physical activity and other lifestyle factors, all factors that have been reported to be associated with mortality risk.

The strengths of this study include the population-based study design, the homogeneity with regard to age and ethnicity, the 97% participation rate in the fracture and mortality evaluation and the—to our knowledge—unprecedented study length. Furthermore, prospective fracture registration through a well-validated method that only includes objectively verified fractures is likely to increase the reliability of our findings. The definition of the menopause using the World Health Organization (WHO) classification[13] and determination of the age at menopause being established at the start of the study, instead of using retrospectively estimated age at the menopause as in most previous studies, must also be regarded as a study strength. Study limitations include the sample size and the number of drop-outs in the bone mass evaluations. As the participation rate in the bone mass evaluation was only 51% (198/390) these data must be regarded as less reliable than the fracture and mortality data. Nevertheless, a participation rate in the BMD measurements of 66% among the women still alive is creditable three decades after the start of the study. It would also have been of interest to evaluate an even lower cutoff value of age at menopause in our study. However, this would have to be based on retrospective estimations of menopause age, with the risk of recall bias. Instead we chose to use only data provided at the actual date of the evaluation and then applied the generally accepted WHO classification when defining age at menopause, after which the women were divided into groups of early and late menopause. It would also have been advantageous to have included preplanned spine radiograms in the evaluation, as many women with osteoporotic spinal fractures never seek medical advice at the time of the fracture.

Considering the restrictions discussed above, we can conclude that a menopause before age 47 is associated with an increased risk of mortality, fragility fractures and osteoporosis at age 77.

Disclosure of interests

None.

Contribution to authorship

OS and MKK were the main authors and were responsible for study design and statistical calculations; MKK also collected the data. HJA was responsible for study design and data collection and co-authored the paper. JÅN performed the statistical calculations.

Details of ethics approval

At the start of the study in 1977, no permission from the institutional review board and no consent form were required; the women were asked to provide oral informed consent. However, later in the course of the study, permission was granted by the ethics committee of the University of Lund, the parent organisation of Skåne University Hospital. Approval date was 25 January 2000 and reference number is LU 625-99.

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Paper II

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Physical activity reduces bone loss in the distal forearm in post-menopausal women – a 25-year prospective study

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Intervention studies have shown that high-intensity training programs with duration of 1–3 years can influence bone mass in post-menopausal women. We wanted to investigate whether moderate physical activity could be associated with reduced post-menopausal bone loss also in the long-term perspective. We evaluated changes in bone mass and bone structure by repeated single-photon absorptiometry measurements of the distal forearm in 91 moderately physically active and 21 inactive women, categorized according to information from questionnaires, from menopause and on average 25 years onwards. Data were calculated with analysis of variance and analysis of covariance tests and presented as means with 95% confidence interval. There were no group differences in bone mass or bone structure at menopause. The mean annual loss in bone mineral content was 1.2% (1.1, 1.3) in the physically active and 1.6% (1.3, 1.8) in the inactive women (after adjustment for menopausal age $P = 0.02$) and the mean decline in a strength index based on bone mass and bone structure was 0.7% (0.6, 0.8) in the physically active and 1.2% (0.8, 1.5) in the inactive women ($P = 0.004$). There were no group differences in the changes in bone structure. Physical activity is also in a long-term perspective associated with reduced post-menopausal bone loss.

Bone strength depends on bone mass, skeletal macro- and micro-architecture, and material properties (Jarvinen et al., 2005; Seeman & Delmas, 2006). All these traits seem possible to influence by physical activity (Uusi-Rasi et al., 1998; Haapasalo et al., 2000; Kaptoge et al., 2003; Martyn-St James & Carroll, 2006; Hind & Burrows, 2007), predominantly during periods with high bone turnover such as growth and adolescence (Hind & Burrows, 2007; Lofgren et al., 2011; Nikander et al., 2010). The skeletal response to physical activity is in generally less pronounced in adulthood (Wallace & Cumming, 2000; Shea et al., 2004; Martyn-St James & Carroll, 2006; Nikander et al., 2010). However, because the post-menopausal years is a specific period with transiently increased bone loss (Recker et al., 2000; Ahlborg et al., 2001), it is plausible that physical activity could exert skeletal effects of biological significance also in this period.

Previous publications have associated high levels of physical activity in post-menopausal women with higher bone mineral density (BMD) (Wallace & Cumming, 2000; Shea et al., 2004; Martyn-St James & Carroll, 2006) and low fracture incidence (Cummings et al., 1995; Feskanich et al., 2002). However, the majority of these studies has been designed as controlled exercise intervention programs with duration of 1–2 years and has not evaluated physical activity and bone mass specifically following menopause. Commonly, the published reports have followed bone mass from a defined chronological age but with menopause occurring at different ages (Wallace & Cumming, 2000; Shea et al., 2004; Martyn-St James & Carroll, 2006, 2008). Although there exist some prospective evaluations of the effect on skeletal traits with follow-up up to 10 years (Kaptoge et al., 2003; Uusi-Rasi et al., 2005, 2006, 2008), none has, to our knowledge, followed a population-based cohort of physically active and inactive women with menopause as baseline and up to ages when fragility fractures become a problem in magnitude.

Therefore, in this prospective observational study, we used menopause as baseline and hypothesized that physical activity would be associated with low post-menopausal bone loss and skeletal benefits of clinical significance in terms of reducing fracture incidence. We asked whether moderate level of physical activity in the post-menopausal period is associated with low post-menopausal bone loss and benefits in bone size.

Material and methods

Our population-based sample included 241 Swedish women aged 48, all white, who were invited in 1977 to participate in a prospective observational study, the study design in detail reported previously (Rannevik et al., 1995; Ahlborg et al., 2001). Excluded at study start were 49 women who were menopausal, taking medications, or had conditions that interfered with bone metabolism, leaving 192 women to enter the study. A further 21 women were excluded during the first 5 years, 17 because of surgical menopause or relocation and 4 due to technical baseline measurement errors. In addition, all 17 women with a history of estrogen treatment and 15 who died were excluded, leaving 139 women followed through their spontaneous menopause in a 12-bone-measurement series.

The present report includes the 112 women (81% of the original 139) who participated at baseline and in either of the last two bone measurements which were performed at age 72 and 77. During the follow-up period, five women (four active and one inactive) had temporarily used corticosteroids for 1–2 years and two (both inactive) used bisphosphonates at age 77. One hundred and two women (91% of 112) attended at least 10 out of the 12 measurements, 7 nine measurements, 2 seven measurements, and 1 six measurements. The last premenopausal measurement, performed no more than 2 years before menopause, was defined as the menopausal measurement and used as baseline (Ahlborg et al., 2001; Ahlborg, 2003). As the women underwent menopause at different chronological ages, the average post-menopausal follow-up period was 25.3 $\pm$ 2.3 years [mean $\pm$ standard deviation (SD)].

The women answered extended lifestyle and physical activity questionnaires at baseline and at age 72 and simplified questionnaires at the other measurements. Months of amenorrhea and general health status were reported in questionnaires and personal interviews by the same research nurse at each measurement. The women were asked to report hours per week of everyday, general physical activity, such as walking, cycling, gardening, and regular physical exercise including all types of sports at four defined time periods: at menopause, 5 and 10 years after menopause, and at age 72. Average physical activity was estimated as the mean of these four periods and a cutoff value of average 30 min of physical activity per day was chosen for dichotomization into one moderately active and one non-active group. The threshold of 30 min represents the recommended minimum level of daily physical activity established in public health recommendations (Haskell et al., 2007).

Menopause was determined retrospectively, using the World Health Organization definition (WHO, 1981), i.e., 12 months of spontaneous amenorrhea and elevated serum levels of follicle-stimulating hormone, representing permanent cessation of menstruation due to the loss of ovarian follicular activity. Follicle-stimulating hormone was analyzed by double antibody radioimmunoassay every 3 months during the first year, then every 6 months until 1 year after menopause, and then yearly (Rannevik et al., 1995; Ahlborg et al., 2001).

Forearm bone mineral content (BMC; mg/cm) and bone mineral apparent density (BMC/cortical area; mg/cm^3) (Hsu et al., 1993) were measured 6 cm proximally of the ulnar styloid process with the same single-photon absorptiometry densitometer, on average, every second year on 12 occasions. A rectilinear scan across the radius and ulna, with a radiation source (^{241}Americium) and a detector moving simultaneously, was used according to the method of Nauclér et al. (1974). The average value of the left and right forearm was used. No long-term drift, determined by measurement of a standardized phantom every second week during the study, was observed (Ahlborg et al., 2001). Due to a replacement of the radiation source in 1980, all measurements thereafter were adjusted by the use of the phantom data. The precision of the single-photon absorptiometry was 1.7%, determined by measurements of the standard phantom, and 4% *in vivo* in double measurements after repositioning of the subjects. The cortical thickness, calculated as the difference between the periosteal diameter and the medullary diameters which were estimated from the graphical representations of the scan, were found to have a coefficient of variation of 8% (Nauclér et al., 1974). The total cross-sectional area, the medullary area and the cortical area can be calculated by assuming the bone to be cylindrical [area = (diameter)^2•π/4]. The cross-sectional moment of inertia was calculated as [(periosteal diameter/2)^4 – (medullary diameter/2)^4]•π/4 and section modulus as [cross-sectional moment of inertia/(periosteal diameter/2)], calculations highly correlative with distal radius strength (Augat et al., 1996). The strength index, taking both the bone mass and skeletal structure into account and proven to correlate with mechanical strength in rat bones (Hsu et al., 1993), was calculated as section modulus • bone mineral apparent density (Ferretti et al., 1996). Body weight and height were measured by standard equipment and grip strength by a Martin vigorimeter® (Heinrich C. Ulrich, Werkstätten für Medizinmechanik, Ulm-Donau, Germany).

Low-energy fractures, sustained after a fall from no greater than the standing position, occurring between menopause and 31 December 2008 (age 79), were identified from patient questionnaires and hospital charts. In the city of Malmo, there is only one hospital, so virtually all fracture patients attended the hospital. The classification system has been used in epidemiological studies for decades and is well validated, as reports have shown that less than 3% of fractures are missed (Bengner, 1987; Jónsson, 1993).

The annual percentage change was calculated for each woman as the ratio of the slope fitted to each woman's repeated measurements divided by the baseline value. ANOVA tests, chi-square tests, Pearson's test, and risk ratio tests were used for group comparisons, and the ANCOVA test was used when adjusting the group differences for menopausal age and post-menopausal levels of estrogen. Separate adjustments for the latter two variables were made, because of a moderate collinearity ($r = 0.45$). The study was approved by the Ethics Committee at Lund University and conducted according to the Declaration of Helsinki.

Results

There were no group differences in bone mass, skeletal geometry, or strength index at baseline (Table 1). In the questionnaire provided at age 72, we found significant group differences in the level of physical activity between physically active and inactive women (Table 2), but no group differences in other lifestyle factors or morbidity (Table 2). Age at menopause and mean post-menopausal serum estradiol levels differed significantly between the two groups.

There was a significant post-menopausal loss in BMC in both physically active and inactive women over the total follow-up period, but the mean annual decrease in BMC was 0.4 percentage points [95% confidence interval (CI) 0.1, 0.6] less in the physically active women (Table 3). The difference remained after adjusting for menopausal age ($P = 0.02$) and mean post-menopausal estradiol level ($P = 0.008$). At age 77, the physically active women had on average 0.5 SD (95% CI 0.1, 1.0) higher BMC than the physically inactive women.

There was also a significant increase in both periosteal and medullary width in both groups over the total follow-up period, but no group differences (Table 3). As

Table 1. Characteristics at age 48

		Active women ($n = 91$)		Inactive women ($n = 21$)		
		Mean	95% CI	Mean	95% CI	*P*-values
Physical activty	h/week	9.0	7.8, 10.2	3.0	1.6, 4.4	0.002
Menarche	Age	14.1	13.8, 14.4	14.0	13.6, 14.4	0.80
		n	%	*n*	%	
Calcium intake	<400 mg/day	16	17.6	4	19.0	0.89
	>400 mg/day	74	81.3	17	81.0	
	Missing	1	1.1	–	–	
Smoking	No	57	63	13	62	0.85
	Yes	32	35	8	38	
	Missing	2	2	–	–	
Number of children	0	9	10	1	5	0.80
	1–3	77	85	20	95	
	>3	4	4	–	–	
	Missing	1	1	–	–	
Breast feeding	No	16	18	2	10	0.37
	Yes	71	78	18	86	
	Missing	4	4	1	5	
Oral contraceptives	No	83	91	18	86	0.45
Previous use	Yes	8	9	3	14	
Workload	Light	74	81	17	81	0.96
at age 45	Moderate	14	15	3	14	
	Heavy	–	–	–	–	
	Missing	3	3	1	5	

Data on lifestyle at age 48 in 91 physically active and in 21 physically inactive women. The data are presented as means with 95 percent confidence interval (95% CI) or as numbers with percentage. No significant differences were found except when comparing duration of physically activity.

a result of the changes in bone mass and bone size, the annual decrease in the strength index was 0.4 percentage points (95% CI 0.2, 0.7) less in the physically active than in the inactive women (Table 3).

The women who were physically inactive during the follow-up period had, in comparison with the physically active women, a risk ratio of 1.1 (95% CI 0.3, 3.2) of sustaining a distal radius fracture and of 1.4 (95% CI 0.6, 3.0) of any incident fragility fracture during the study period (Table 4).

Discussion

This report is, to our knowledge, the first one to use menopause as baseline when prospectively evaluating the association between regular, moderately intense, and non-specified physical activity and post-menopausal bone loss. This is probably of clinical relevance because there is a transient increase in bone loss following menopause. The finding that bone mass was not higher in the active women at baseline and that the annual post-menopausal bone loss was lower in these women implies that a consistent level of at least moderate physical activity after menopause could be one long-term prevention strategy for reducing bone loss.

There are studies with a higher level of evidence that evaluate the effect of physical activity on the skeleton in the post-menopausal period, but the majority of these span at best for 24 months, include heterogeneous populations as regards age and years since menopause, and predominantly use intervention with impact activities (Wallace & Cumming, 2000; Shea et al., 2004; Martyn-St James & Carroll, 2006, 2008), which are known to be osteogenic (Lanyon & Rubin, 1984; Lanyon, 1992). These studies infer that high-impact activity may at best reach a 1–3% increase in BMD, benefits with questionable relevance in terms of fracture reduction (Melton et al., 1993; Marshall et al., 1996; Johnell et al., 2005). There are also prospective observational studies that have associated physical activity with reduced bone loss (Greendale et al., 1995; Uusi-Rasi et al., 2006; Uusi-Rasi et al., 2008) and lower fracture risk (Feskanich et al., 2002), but principally, observational studies cannot prove causality; physically active women could be more likely to be active because of their larger muscle mass (and bone mass), present before the activity was started rather than being the result of the activity. However, this report was able to show that there were no differences in bone mass, skeletal geometry, grip strength, or body mass index at baseline, thus reducing the risk of selection bias. But even so, this study cannot prove causality. Healthier individuals may choose to be more active, while less healthy persons exercise less because of their illness. However, we found no

Table 2. Characteristics at age 72

		Active women ($n = 91$)		Inactive women ($n = 21$)		
		Mean	95% CI	Mean	95% CI	*P*-values
Present physical activity	h/week	8.2	6.9, 9.4	1.2	0.5, 1.9	<0.001
Average post-menopausal physical activity	h/week	8.7	7.6, 9.8	2.0	1.4, 2.5	<0.001
Menopause	Age	51.8	51.3, 52.2	53.2	52.4, 54.1	0.05
Post-menopausal serum estradiol level	pmol/L	89.5	83.9, 95.1	75.7	66.3, 85.1	0.03
		n	%	*n*	%	
Calcium intake	<400 mg/day	42	46	10	48	0.90
	>400 mg/day	49	54	11	52	
Smoking	Never	57	63	13	62	0.84
	Former	25	27	3	13	
	Current	9	10	4	19	
	Missing	–	–	1	5	
Alcohol consumption	None	11	12	5	24	0.20
	Occasional	59	65	14	67	
	Regular	21	23	2	10	
Coffee consumption	None	3	3	–	–	0.88
in cups per day	1–3	49	54	11	52	
	>3	37	41	10	48	
	Missing	2	2	–	–	
Workload at age 45	Light	71	78	14	67	0.23
	Moderate	19	21	7	33	
	Heavy	–	–	–	–	
	Missing	1	1	–	–	
Specific symptoms						
Cardiovascular	Yes	8	9	2	10	0.92
	No	83	91	19	90	
Pulmonary	Yes	8	9	1	5	0.54
	No	83	91	20	95	
Gastrointestinal	Yes	10	11	3	14	0.68
	No	80	89	18	86	
Musculo-skeletal	Yes	26	29	9	43	0.20
	No	65	71	12	57	
Fatigue	Yes	11	12	4	19	0.40
	No	80	88	17	81	
		Mean	95% CI	Mean	95% CI	
Number of operations undergone		1.4	1.2, 1.7	1.6	1.0, 2.1	0.58
Number of drugs taken		1.5	1.1, 1.8	2.0	1.2, 2.7	0.21

Follow-up characteristics reported at age 72 in 91 physically active and 21 physically inactive women. The age 72 data were chosen because the extended questionnaire was used at this point. The data are presented as means with 95 percent confidence interval (95% CI) or as numbers with percentage. No significant group differences were found except when comparing duration of physical activity, menopausal age, and post-menopausal estradiol levels.

group differences in prevalence of diseases or medication or lifestyle that could explain the group differences in the changes in bone mass. Furthermore, because our conclusions remained after adjustments for menopausal age and post-menopausal serum estradiol levels, it seems reasonable that the levels of physical activity had a substantial impact on the bone loss in our study, even though it was not designed to include specific osteogenic loads.

With the restrictions discussed earlier in mind, our data imply that moderate physical activity following menopause is associated with long-term benefits in cortical, non-weight-loaded bone of 0.5 SD at age 77. According to previously reported studies, this would reduce the fracture risk by around 25% (Marshall et al., 1996; Johnell et al., 2005). However, we found no statistically significant reduction in fracture rate in the physically active women, although the nonsignificant 39% lower incidence in fragility fractures in this cohort at least does not oppose the hypothesis that physical activity is associated with low fracture risk. Because larger studies have reported an obvious correlation between high level of physical activity and low fracture risk in elderly (Feskanich et al., 2002; Michaelsson et al., 2007), the inability to reproduce this association in

Table 3. Changes in bone mass and bone structure during the follow-up period

	Measurement at MP					Average annual changes					
	Physically active women ($n = 91$)		Physically inactive women ($n = 21$)		*P*-values	Physically active women ($n = 91$)		Physically inactive women ($n = 21$)		*P*-values adjusted for age at menopause	*P*-values adjusted for post-menopausal estradiol levels
	Mean	95% CI	Mean	95% CI		Mean	95% CI	Mean	95% CI		
Anthropometrics											
Height (cm)	164	(163, 165)	163	161, 165	0.39	–0.1	–0.1, –0.1	–0.1	–0.1, –0.0	0.57	0.33
Weight (kg)	64.5	62.3, 66.7	62.5	57.7, 67.3	0.44	0.3	0.2, 0.4	0.4	0.0, 0.7	0.61	0.72
BMI (kg/m^2)	24.0	23.2, 24.8	23.5	21.8, 25.3	0.65	0.5	0.4, 0.6	0.5	0.0, 0.9	0.99	0.78
Grip strength (kp/cm^2)	0.75	0.72, 0.79	0.78	0.71, 0.85	0.49	–1.7	–1.8, –1.5	–2.1	–2.5, –1.8	0.16	0.04
Bone mass											
BMC (mg/cm)	725	706, 743	727	697, 758	0.90	–1.2	–1.3, –1.1	–1.6	–1.9, –1.3	0.02	0.008
BMAD (mg/cm^3)	757	738, 775	786	733, 838	0.38	–1.8	–1.9, –1.7	–2.0	–2.2, –1.7	0.22	0.39
Skeletal structure											
Periosteal diameter (mm)	13.0	12.8, 13.2	13.0	12.4, 13.5	0.86	0.5	0.5, 0.6	0.5	0.4, 0.6	0.56	0.54
Medullary diameter (mm)	6.7	6.5, 7.0	6.8	6.2, 7.4	0.87	0.8	0.7, 0.9	0.9	0.6, 1.2	0.62	0.46
Cortical thickness (mm)	6.3	6.1, 6.4	6.2	5.9, 6.4	0.57	0.3	0.2, 0.4	0.2	–0.1, 0.5	0.37	0.23
Skeletal strength											
CSMI (cm^4)	0.13	0.13, 0.14	0.13	0.11, 0.15	0.88	2.4	2.1, 2.7	2.4	1.8, 3.0	0.49	0.64
Section modulus (cm^3)	0.20	0.19, 0.21	0.20	0.18, 0.22	0.82	1.7	1.5, 1.9	1.6	1.2, 2.1	0.46	0.55
Strength index	151	145, 157	153	139, 168	0.94	–0.7	–0.8, –0.6	–1.2	–1.5, –0.9	0.004	0.002

Anthropometrics, bone mass, skeletal structure and bone strength of the distal radius measured by single-photon absorptiometry at menopause (MP) and repeatedly during the study period, presented with average annual changes. The data are presented as means with 95 percent confidence interval (95% CI). Group comparisons are computed with analysis of covariance and separately adjusted for menopausal age and average post-menopausal estradiol levels.

BMAD, bone mineral apparent density; BMC, bone mineral content; BMI, body mass index; CSMI, cross-sectional moment of inertia.

Table 4. Fracture incidence

	Physically active women ($n = 91$)	Physically inactive women ($n = 21$)
Distal radius fractures		
Numbers of women (%)	18 (20)	5 (31)
Fracture rate per 10^3 person-years	7.5	8.3
Risk ratio (95% CI)		1.1 (0.3,3.2)
Fragility fractures		
Numbers of women (%)	28 (31)	9 (43)
Fracture rate per 10^3 person-years	11.3	15.7
Risk ratio (95% CI)		1.4 (0.6,3.0)

The number of women with distal radius fracture and any fragility fracture sustained between menopause and age 79 and fracture rate per 10^3 person-years in physically active and inactive women. The risk ratio with 95 percent confidence interval (95% CI) is presented for physically inactive women in comparison with active women.

our study may be the result of the relatively small sample size and consequently limited statistical power, hence a possible type II error. As the weight-loaded skeletal parts and trabecular bone are more susceptible to mechanical impact than cortical bone (Lanyon & Rubin, 1984; Lanyon, 1992) and because the numerous previous studies that have documented the skeletal benefits of physical activity have been carried out with dual X-ray absorptiometry (DXA) measurements of the femoral neck and lumbar spine (Wallace & Cumming, 2000; Shea et al., 2004; Martyn-St James & Carroll, 2006, 2008), we can only assume that the effects observed in the cortex of the distal forearm in the current report would at least not be smaller in trabecular and weight-loaded bone where fractures are more frequent. Another weakness is that our study was not a randomized controlled trial, thus not allowing conclusions regarding causality between physical activity and bone loss. Our study is observational, and levels of physical activity were estimated in questionnaires. It would have been advantageous to include an objective registration of duration, intensity, and type of the performed activities. However, with 2–3 decades of follow-up, a randomized study would be virtually impossible to maintain. Another concern is that as bone mass decreases with advancing ages, edge detection for calculating periosteal and endosteal width with the single-photon absorptiometry could be impaired. It would therefore have been preferable to use DXA for evaluating axial skeletal regions and peripheral computed tomography (pQCT) in order to assess bone geometry more specifically, but these techniques were not available during the first decade of the study.

The advantages include the well-defined population-based female cohort of white, north European women without diseases known to interfere with bone metabolism and with baseline measurements conducted close to menopause. The repeated measurements with the same apparatus with no long-term drift, with measurements and analyses done by the same technician, a high attendance rate, and an accurate estimation of menopause are other advantages. Finally, conducting a similar study would hardly be feasible today, given the introduction of medication for prevention of osteoporosis that would interfere in the natural course of post-menopausal skeletal changes.

In summary, this study infers that bone loss following menopause is less in moderately physically active than in inactive women. We therefore suggest that moderate physical activity could be recommended to menopausal women as a strategy to prevent bone loss.

Perspectives

This prospective study documents the long-term skeletal benefits of physical activity. Previous studies on the effects of physical activity in elderly people have mainly been controlled exercise intervention programs of a duration of 1–2 years, whereas the present report can show that even moderate levels of physical activity help in preserving bone mass up in the ages where fractures begin to increase exponentially. In the light of these findings, we suggest post-menopausal women be recommended physical activity, not only to gain fall-preventive neuromuscular benefits but also in order to reduce the age-related bone loss. We look forward to reading prospective long-term reports of larger materials where also fracture incidence could be evaluated in relation to activity levels.

Key words: physical activity, bone loss, bone mass.

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Paper III

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Original Article

Hylonome

Changes in forearm bone mass and bone size after menopause – A mean 24-year prospective study

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Abstract

Objective: Bone loss and periosteal expansion is found after menopause. The accelerated early postmenopausal bone loss is not permanent but if the same accounts for the periosteal expansion is unknown. **Methods:** Bone mineral density (BMD) and skeletal structure of the distal forearm were followed from menopause and on average 24 years (range 18-28) by single-photon absorptiometry at 12 occasions in a population-based sample of 81 Caucasian women with no medication or disease affecting bone metabolism. A Strength Index based on areal BMD and bone structure was calculated. Postmenopausal serum-estradiol levels and incident distal radius fractures were registered. Data are presented as means with 95% confidence interval (95% CI). **Results:** The annual BMD loss in three periods, 0-8, 8-16 and 16-28 years after menopause, was 2.0% (1.6, 2.4), 1.0% (0.6, 1.4) and 1.0% (0.7, 1.3), respectively. The annual periosteal expansion was 1.0% (0.8, 1.3), 0.0% (-0.3, 0.3) and 0.0% (-0.2, 0.2), respectively. Mean post-menopausal oestrogen levels correlated moderately with annual loss in aBMD ($r=0.51$, $p<0.001$) but less with the annual changes in bone width ($r= -0.22$, $p=0.06$). **Conclusion:** Postmenopausal periosteal expansion in the distal forearm seems to occur only in the first postmenopausal decade.

Keywords: Bone Loss, Bone Mineral Density, Bone Size, Postmenopausal, Oestrogen

Introduction

Post-menopausal bone loss follows a pattern with an early oestrogen-dependent phase of rapid decline in bone mass followed by an age-related bone loss at a constant, slower rate throughout ageing[1,2]. Our group has previously reported that the early post-menopausal bone loss is associated with an increased bone width in the forearm up to age 67[3], and the same phenomenon is shown in the femoral neck, both in cross-sectional[4-7] and longitudinal[8,9] studies. The increase in bone size would in mechanical models partially preserve bone strength through increased resistance to bending forces. Small bone size has also been independently associated with fractures in both the femoral neck[10] and in vertebrae[10-12]. In other words, both material qualities such as the tissue mineral content and strength properties such as the Young's modulus are of importance for the skeletal ability to resist a fracture during a fall[13]. Whether the periosteal expansion exists only in association with the oestrogen-dependent fast bone loss in the early postmenopausal period or continues also into higher ages when fragility fractures become a problem in magnitude is still unknown since most studies on changes in bone width are either cross-sectional[4-6] or of too short follow-up[3,7-9,14,15] for this particular question.

While it is well established that oestrogen suppresses bone resorption[16,17] and bone turnover[18], bone size in humans has not been shown to be affected by falling oestrogen levels, although the hormone-related post-menopausal bone loss is accompanied by periosteal expansion.

Several reports have forwarded that the skeletal response to external stimuli, such as mechanical load and changes in hormonal status, is most pronounced during periods with high bone turnover such as during growth[19-21]. The early postmenopausal phase is another period in life with accelerated bone turnover[1,3]. Therefore, periosteal expansion could possi-

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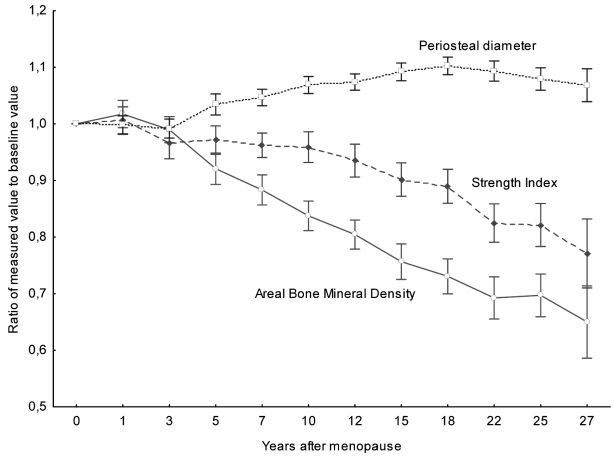

Figure 1. Mean relative changes in periosteal diameter, areal bone mineral density (aBMD) and Strength Index at the cortical site of the distal radius in 81 women followed from menopause and for a mean 24 years (range 18 to 28). In comparison with baseline values, aBMD became significantly lower 5 years after menopause, periosteal diameter significant wider 5 years after menopause while Strength Index did not become significantly lower until 12 years after menopause. Data are presented as means with standard errors.

bly be more pronounced in the early than in the remote postmenopausal period.

With this background, we extended our previous report[3] for another decade with the following aims: (i) to evaluate if periosteal expansion is more pronounced in the early postmenopausal period with a fast loss in BMD than in a long-term perspective; (ii) to investigate whether the skeletal changes are primarily associated with the postmenopausal levels of oestrogen or the peri-menopausal decline in oestrogen levels; and (iii) as the study was not powered to include fracture as endpoint variable, report incident fractures in relation to quartiles of BMD and Strength Index only as descriptive data.

Methods

In 1977 we invited a population-based sample of 48-year-old Caucasian women (n=241) to participate in this prospective study that aimed to follow women who were non-menopausal at baseline[1,22]. Forty-nine women were excluded at baseline because they were peri- or postmenopausal or had conditions or medications interfering with bone metabolism, leaving 192 women with cyclic menstrual bleedings eligible to enter at study start 1977-1978. During the first 5 years, 17 withdrew because of surgically induced menopause or relocation, 4 were omitted because of baseline technical measurement errors, 17 owing to menopausal oestrogen treatment and 8 died, resulting in 146 remaining women who were followed through their spontaneous menopause. BMD was measured on 12 occasions, initially every second year and thereafter at intervals of 3 to 5 years. The average attendance rate was 11.4 (range 7-12) measurements. During the total 28-year follow-up period, another 22 died, 5 relocated, 7 received corticosteroids or anti-resorptive osteoporosis therapy, 29 declined participation for personal reasons or diseases and 7 had to be excluded due to technical measurement errors at the 12th and last measurement at age 76. In sum, 81 women were followed throughout the entire study period, constituting the cohort of this report. Since menopause occurred at different ages with an average menopausal age of 52 (range 48-57), the postmenopausal follow-up period was a mean 24 years (range 19-28).

Menopause was estimated according to the criteria established by the World Health Organization[23], i.e. permanent cessation of menstruation due to the loss of ovarian follicular activity. The onset of menopause was therefore determined retrospectively when 12 months of spontaneous amenorrhoea was reported, in conjunction with elevated serum levels of follicle-stimulating hormone. Follicle-stimulating hormone was analysed by double-antibody radioimmunoassay[24,25] every three months during the first year, then every six months until one year after menopause, and then yearly. Serum level of estradiol was also determined after ether extraction every year until 8 years after menopause as described previously[24]. As serum levels of estradiol in this cohort decreased during the first 3 postmenopausal years, but not after this[24], the postmenopausal estradiol level was defined as the mean value from 3 to 8 years postmenopausal. The duration of amenorrhoea and general

Variable	At menopause	At age 76 years	Annual changes during the entire study period	Annual changes 0-8 years after menopause	Annual changes 8-16 years after menopause	Annual changes 16-28 years after menopause
Bone structure						
Periosteal diameter (mm)	13.0 (12.8, 13.3)	14.3 (14.0, 14.5)	**0.4% (0.4, 0.4)**	**1.0% (0.8, 1.3)**	0.0% (-0.3, 0.3)	0.0% (-0.2, 0.2)
Medullary diameter (mm)	6.8 (6.5, 7.1)	7.7 (7.5, 8.0)	**0.7% (0.6, 0.8)**	**1.2% (0.8, 1.6)**	0.3% (-0.3, 0.9)	0.4% (-0.2, 1.1)
Cortical thickness (mm)	6.3 (6.1, 6.4)	6.6 (6.4, 6.7)	**0.2% (0.1, 0.3)**	**1.0% (0.5, 1.4)**	-0.3% (-0.8, 0.1)	-0.3% (-0.9, 0.2)
Bone mass						
Bone mineral content (mg/cm)	727 (709, 744)	521 (498, 545)	**-1.1% (-1.2, -1.0)**	**-1.1% (-1.5, -0.8)**	**-1.1% (-1.5, -0.7)**	**-1.0% (-1.3, -0.8)**
Areal bone mineral density (mg/cm^2)	558 (547, 570)	366 (350, 383)	**-1.4% (-1.5, -1.3)**	**-2.0% (-2.4, -1.6)**	**-1.0% (-1.4, -0.6)**	**-1.0% (-1.3, -0.7)**
Bone mineral apparent density (mg/cm^3)	758 (737, 778)	467 (446, 488)	**-1.5% (-1.6, -1.4)**	**-2.7% (-3.3, -2.2)**	**-0.7% (-1.3, -0.2)**	**-0.7% (-1.1, -0.4)**
Bone strength						
Cross-sectional moment of inertia	0.14 (0.13, 0.14)	0.19 (0.18, 0.21)	**1.8% (1.6, 2.0)**	**4.7% (3.4, 6.0)**	-0.1% (-1.6, 1.3)	0.2 (-1.4, 1.8)
Section modulus (cm^3)	0.20 (0.19, 0.21)	0.26 (0.25, 0.28)	**1.3% (1.1, 1.4)**	**3.3% (2.4, 4.2)**	-0.1% (-1.1, 0.9)	0.0 (-1.0, 1.1)
Strength index	152 (145, 159)	120 (115, 127)	**-0.7% (-0.8, -0.6)**	-0.2% (-0.6, 0.3)	**-1.1% (-1.6, -0.6)**	**-1.0 (-1.4, -0.4)**

Table 1. Skeletal structure, bone mass and bone strength at the cortical site of the distal radius in 81 women who were followed through their spontaneous menopause with repeated measurements with a mean postmenopausal follow-up period of 24 years (range 18–28). Data are presented as means with 95% confidence interval (95% CI) and at baseline (menopause) and follow-up (age 76 years) in absolute values and as annual changes in per cent in relation to the menopausal values.

health were reported through a questionnaire and personal interviews conducted by the department's research nurses.

Bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2) in the forearm were measured at a cortical site 6 cm proximal to the styloid process of the ulna every other year by single-photon absorptiometry (SPA), according to the method described by Nauclér et al.[1,3,26]. The tissue mineral content, expressed as the bone mineral apparent density (in milligrams per cubic centimetre), was calculated as the bone mineral content divided by the cortical area[3]. The radii and ulnae of both right and left forearm were scanned and one average value for all four bones was calculated for both aBMD and bone width. The same densitometer was used throughout the study, and no long-term drift was detected at measurements of a standardized phantom every other week[1,3]. Since the radiation source was replaced in 1980, all measurements thereafter were adjusted with the use of the data from the phantom. The coefficient of variation of the bone mass measurements on single-photon absorptiometry was 1,7% with the standard phantom and 4% *in vivo* determined by repeated measurements after repositioning of the measured subjects. The co-efficient of variation for bone width was 1,6%, estimated by the phantom data[27]. The cortical thickness calculated as the difference between the periosteal diameter (bone width) and the medullary diameters which were estimated from the graphical representations of the scan, has been found to have a coefficient of variation of 8%[26].

Two strict mechanical calculations, cross-sectional moment of inertia and the section modulus, and one Strength Index taking both bone tissue density and bone structure into account, were calculated as reported in our previous publication[3]. In mechanical terms, the section modulus represents the bone's resistance to static bending forces.

Variable	No. of women with distal radius fracture	Fracture rate per 1000 person-years
aBMD		
Quartile 1	4	7.4
Quartile 2	3	5.1
Quartile 3	4	8.0
Quartile 4	2	3.6
Strength Index		
Quartile 1	7	14.3
Quartile 2	2	3.6
Quartile 3	3	5.3
Quartile 4	1	1.7

Table 2. Number of women with distal radius fractures during the mean 30-year (range 25-34) follow-up period in relation to quartiles of areal bone mineral density (aBMD) and Strength Index at menopause. Quartile 1 represents the quartile with lowest values.

Distal radius fractures sustained after a fall from no higher than the standing position between menopause and 2011, a follow-up period of mean 30 years (25-34), were identified from patient questionnaires and the hospital archives and databases. In our city there is one hospital, so virtually all fracture patients

attend the emergency unit, and the classification system has been used in epidemiological studies for decades and is well validated, as reports have shown that less than 3% of fractures are missed[28,29].

At study start, no permission from the institutional review board and no consent form were required, but the women were informed and gave oral consent. Written permission was granted by the ethics committee of Lund University in 1999, when written informed consent was also obtained from each individual. The study has been conducted in accordance with the norms of the Helsinki Declaration. Statistical processing has been carried out using STATISTICA software version 7.1 (StatSoft). Data are presented as means with 95% confidence interval (95% CI). In order to compare changes in bone parameters during different phases of the postmenopausal period, the follow-up period was divided into three separate intervals, 0-8, 8-16 and 16-28 (mean follow-up 24) years after menopause. The last measurement before menopause, performed 0-2 years pre-menopause, was denoted as the baseline measurement. The annual percentage change was calculated for each woman as the ratio of the slope fitted to that woman's repeated measurements divided by the baseline value. In Figure 1, the relative change represents the ratio of the observed value at each time point relative to each individual's baseline value. Student's t-test was used to determine at what point the specific measurements differed significantly from baseline. Linear regression equations were used to examine the association between decline in oestrogen from pre- to postmenopausal levels and the postmenopausal oestrogen levels with aBMD and periosteal and medullary changes. Incident distal radius fractures are only presented in relation to baseline quartiles of aBMD and Strength Index.

Results

Bone traits at menopause, at age 76, and annual changes during the total follow-up period and in the periods 0-8, 8-16 and 16-28 years after menopause are presented in Table 1 and Figure 1. In comparison with baseline, BMD became significantly lower 5 years after menopause, periosteal diameter significantly wider 5 years after menopause while the Strength Index only became significantly lower 12 years after menopause (Figure 1). The annual loss in aBMD during the periods 0-8 years, 8-16 years and 16-28 years after menopause was 2.0% (1.6, 2.4), 1.0% (0.6, 1.4) and 1.0% (0.7, 1.3), respectively, and the annual periosteal expansion 1.0% (0.8, 1.3), 0.0% (-0.3, 0.3) and 0.0% (-0.2, 0.2), respectively. As a result, the Strength Index was virtually unchanged during the period 0-8 years, with an annual decrease of 0.2% (-0.3, 0.6), after which there was a significant decrease in period 8–16 years by 1.1% (0.6, 1.6) and in period 16-28 years by 1.0% (0.4, 1.4) (Table 1).

Serum estradiol levels were stable around the mean value of 88.3 pmol/L (95% CI 82.8 to 93.8, SD 24.1) 3 to 8 years after menopause and correlated moderately in period 0–8 years with the annual change in aBMD ($r=0.51$, $p<0.001$) but to a lesser degree with the periosteal diameter ($r=-0.22$, $p=0.06$) (Figure 2). There was no correlation between the decline from pre- to postmenopausal oestrogen levels and the annual change in periosteal diameter ($r=0.17$, $p=0.18$) and a weaker correlation with annual changes in aBMD ($r=-0.37$, $p<0.01$). There was no correlation between postmenopausal serum estradiol levels or the decline in oestrogen levels and annual change of medullary diameter.

Thirteen women sustained incident fragility-related fractures of the distal radius. Descriptive fracture data in relation to quartiles of baseline aBMD and Strength Index are presented in Table 2.

Discussion

We have previously reported that increased bone loss in the distal forearm following menopause is accompanied by periosteal expansion which partially preserves bone strength[3]. The present study suggests that the periosteal expansion in the distal forearm is an impermanent phenomenon, found only in the early postmenopausal period in conjunction with the previously reported accelerated postmenopausal bone loss[1,3] and not in the higher ages when fragility fractures are more frequent. In other words, this structural adaptation of the skeleton could possibly counteract bone loss in the first decade following menopause but not in the remote post-menopausal period.

Changes in bone size have been seen in cross-sectional studies comparing differences between men and women or comparing cohorts stratified according to age, indicating a wider femoral neck[7] and increased bone size in the tibia[6,30] in older people. Also longitudinal data support periosteal expansion of the femoral neck with advancing age, as presented by Uusi-Rasi et al in prospective studies of up to 10 years duration[8,9] where Hip Structure Analyses of DXA measurements[7] of post-menopausal women was used. The forerunner of the current report presents the longest follow-up, as 112 women were followed with repeated SPA measurements from menopause up to age 67 and found a periosteal expansion of 10% of the distal forearm[3]. There are also contradictory prospective data as Szulc et al[14] found lower periosteal apposition in the distal radius in post-menopausal than in pre-menopausal women in their seven-year study of 821 women aged 31-89 years. Also Uusi-Rasi et al found no periosteal expansion in the femoral neck in their 9-year prospective study[15]. However, the study by Ahlborg is the only one to have analyzed periosteal expansion across and on average 15 years after menopause in a homogeneous cohort, whereas other authors have described general changes in bone structure in post-menopausal women regardless of age, and not related to time passed since menopause. Consequently, when aggregating women from a wide span of ages and with varying number of years passed since menopause, the effects of a temporarily increased periosteal expansion in the early post-menopausal period may be obscured. Furthermore, the intrinsic difficulty of detecting subtle dimensional changes in three-dimensional bones must not be underestimated. All bone measurements methods available today are afflicted with limitations as regards image quality and

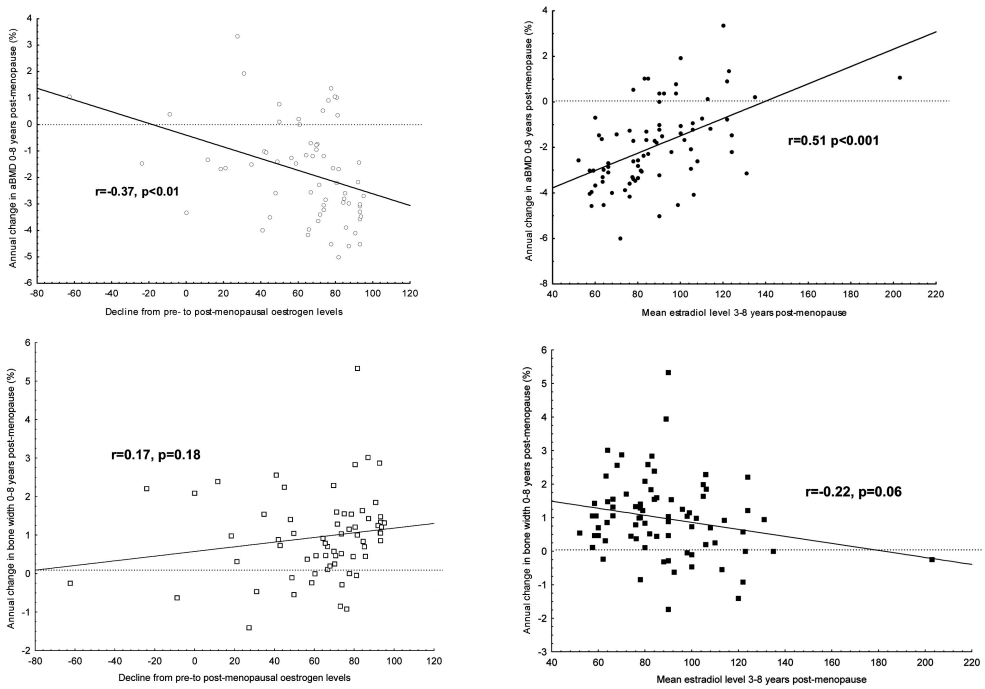

Figure 2. Correlation between post-menopausal decline in serum estradiol and the stable mean estradiol levels 3-8 years post-menopause with annual changes in periosteal diameter and areal bone mineral density (aBMD) at the cortical site of distal radius in 81 women followed from menopause during the period 0 to 8 years after menopause. This period was chosen as the period with significant annual changes in aBMD and periosteal expansion. aBMD correlates moderately with estradiol levels and to a lesser degree with the post-menopausal drop in estradiol levels. Periosteal expansion showed a trend of correlation estradiol levels but not with the postmenopausal drop in estradiol levels.

subject positioning and calculations are based on the assumption that the bone is cylindrical and symmetrical in its thickness.

The fast postmenopausal bone loss in the early postmenopausal period which is associated with a decline in oestrogen and mediated mainly through resorption at the endosteal surface and in Haversian canals[5,6,14,31-33], is well documented[1,34]. However, bone strength depends not only on the material properties but also on the structural characteristics of the skeleton. An increased bone size would counteract a diminished bone density and partially preserve bone strength. If the cortical shell is placed farther away from the long axis of the bone, the resistance of the bone to bending and torsional forces improves. Strict mechanical calculations such as the cross-sectional moment of inertia, which is highly correlated with the strength of the distal radius[35], and the section modulus, a measure of the ability to withstand bending and torsional forces[36], represent the geometrical contributions of bone strength. In this study, the section modulus increased by about 30% during the follow-up. Hypothetically, if no periosteal expansion had occurred, the section modulus would instead have decreased because of the medullary expansion following the bone loss. In our material, we found an average annual increase of 3.3% in the section modulus in the first 8-year interval following menopause and thereafter, no significant changes up to age 76. This is somewhat discordant with other authors who have reported that the section modulus in the femoral neck decreases after menopause[9,15] or is maintained until age 60 and thereafter declines[7].

Periosteal expansion is probably of clinical relevance, since small bone size has been independently associated with fractures in both the femoral neck[10] and in vertebrae[10,12]. The Strength Index is an estimate that takes not only bone structure but also bone mass into account and hypothetically could predict fracture risk better than BMD alone[3] although there is no

statistical evidence of its usefulness in fracture prediction.

There are several plausible explanations for the periosteal expansion. The reduction in oestrogen levels or the absolute low stable oestrogen levels after menopause may result not only in the loss of BMD, but also in periosteal expansion, since oestrogen has been shown to inhibit periosteal bone formation in experiments in rats[37]. Our data indicate that there could be an association with oestrogen levels, even if this is speculative since we found only trends in the correlation analyses. Any influence of oestrogen on periosteal expansion must be regarded as low since postmenopausal oestrogen levels only explained 4.8% of the variance in periosteal expansion during the first 8 postmenopausal years. Another possibility is that bone loss on the endocortical surface causes increased mechanical stresses in the bone tissue, in turn stimulating periosteal bone formation; another hypothesis not possible to test by our study design. The complexity of exploring any relationship between changes in the endosteal and periosteal surfaces should not to be underestimated for several reasons. One may be that cortical bone loss occurs by intracortical remodeling throughout the cortex, not only on the inner aspect. If so, it could not be expected that changes in endosteal and periosteal surfaces would correlate. Furthermore, our mechanical calculations are based on the assumption that the distal radial shaft and the medullary cavity are cylindrical, which we know are approximations that could influence our interpretations of changes in medullary and periosteal width. In addition, all bone scanning techniques today have limitations in detecting small differences in bone dimensions, as discussed above more extensively. And finally, one interpretation could be that the events on the endocortical and periosteal surfaces are independent, not codependent.

It has previously been shown that BMD is one of the best predictors of fractures at the measured site[38]. For this reason, we included only fractures of the distal radius in our analysis, with the knowledge that we measured a cortical region while the reported distal radius fractures occurred in the metaphyseal region. Since both BMD and bone size are independently associated with fractures[10-12,39] and since both traits contribute to bone strength, we combined the tissue-level strength (BMD) with the skeleton's resistance to bending and torsion (section modulus) into a Strength Index. Our descriptive fracture data also seem to show a more marked preponderance of fractures among women in the lowest quartile of Strength Index than in the lowest quartile of BMD. The Strength Index may thus be a usable tool for the prediction of fractures, although further, larger studies are required for statistical evidence.

The advantages of this study include the follow-up period of on average 24 years, the use of menopause as baseline and the homogeneous population-based cohort of Caucasian women without diseases or medications interfering with the skeleton. The use of menopause as baseline is mandatory when trying to answer our hypotheses and a similar study could hardly be conducted today after the introduction of anti-resorptive osteoporosis therapy. Menopause was determined accurately according to the WHO definition and bone mass and bone structure could then be followed through the spontaneous menopause with the same scanner with no long-term drift. The mean 11.4 measurements per woman and the long follow-up period enabled us to calculate individual slopes with better precision than delta values.

The limitations include the small sample size which was not powered for evaluating incident fractures and the use of single-photon absorptiometry in the distal forearm instead of modern scanners and measurements of both axial and appendicular regions. However, such scanning techniques were not available at study start. Measurement reservations include difficulties in capturing very small changes, especially if edge detection may be hampered by changes in fat content in the upper extremity and if bone loss occurs with not only resorption at the endosteal surface but also at the outer surface. We cannot rule out the risk of a type two-error, since discrete changes in small sample sizes may be obscured by errors in measurement,

In spite of these limitations, we suggest that periosteal expansion in the distal forearm is not a permanent phenomenon but found only in the first decade after menopause whereas the loss in BMD continues also in the remote postmenopausal period.

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Low BMD is an independent predictor of fracture and early menopause of mortality in post-menopausal women – A 34-year prospective study

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ABSTRACT

Objective: Identify risk factors for fragility fractures and mortality in women aged 48.
Study design: Prospective population-based observational study on 390 white north European women aged 48 at study start. At study start, we measured bone mineral density (BMD) by single-photon absorptiometry (SPA) in the distal forearm, anthropometry by standard equipment and registered menopausal status, health and lifestyle factors. Menopause before age 47 was defined as early menopause. Incident fragility fractures and mortality were recorded until the women reached age 82. Potential risk factors for fragility fracture and mortality were evaluated with Cox's proportional hazard regression analysis. Data are presented as risk ratios (RR) with 95% confidence intervals in brackets.
Main outcome measures: Incidence of fragility fractures and mortality.
Results: In the univariate analysis, low BMD and early menopause predicted fractures. In the multivariate analysis, only BMD remained as an independent risk factor with a RR of 1.36 (1.15, 1.62) per standard deviation (SD) decrease in baseline BMD. In the univariate analysis, early menopause and smoking predicted mortality, and remained as independent risk factors in the multivariate analysis with RR 1.62 (1.09, 2.39) for early menopause and 2.16 (1.53, 3,06) for smoking.
Conclusions: Low BMD at age 48 is an independent predictor for fragility fractures. The predictive ability of early menopause is at least partially attributed to other associated risk factors. Early menopause and smoking were found in this study to be independent predictors for mortality.

1. Introduction

Published literature has identified a number of potential risk factors for fragility fractures [1,2]. Our research group has previously specifically evaluated the predictive ability of early menopause in a prospective observational cohort study over 34 years where we reported that menopause before age 47 is associated with an increased risk of osteoporosis, fragility fractures and higher mortality [3]. This concords with the body of evidence for low bone mineral density (BMD) and higher fracture incidence in women with early menopause, as summarised by Gallagher in 2007 [4]. Also the higher mortality risk in women with early menopause that we found in our previous study is supported in the literature, as presented by Shuster et al. in a review from 2009 [5]. However, we did not investigate other risk factors than menopause, whether early menopause was independently associated with fragility fractures and mortality, or if the effect could be attributed to other menopause-related risk factors.

Some long-term studies suggest a fading influence of early menopause with increasing age [6–8]. If so, this could be related to the fact that the rapid oestrogen-associated bone loss in the first post-menopausal decade is replaced by a slower age-dependent bone loss [9] and that an increasing number of other risk factors for low BMD and fracture appear in the old woman, eventually diminishing the effect of having an early menopause. The discordance between different studies could be due to methodological issues such as cross-sectional study designs, a retrospective definition of menopausal age with the risk of recall bias, and short follow-up periods [4,6–8]

An ideal study to estimate the long-term effect of risk factors for fractures and mortality should use a population-based prospective design and follow a homogeneous population of individuals from a specified baseline through several decades. The Malmo perimenopausal study is one such study that follows women from age 48 until age 82, with continuous registration of fracture and mortality data.

In this study our primary aim was to identify risk factors for fragility fractures in the postmenopausal period and determine

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whether these remained independent predictors when other identified risk factors were taken into account. Secondly, we wanted to identify independent risk factors for mortality in postmenopausal women.

2. Methods

As described previously, 390 women aged 48 entered this prospective observational study in 1977 [3]. All were white north European female residents of the city of Malmo, Sweden, born during the latter half of 1929 and selected from the city population records. At study start we registered a set of lifestyle parameters which have all been reported to affect BMD or fracture incidence, such as menopausal status [4], age at menarche [10–12], parity [13], breastfeeding [13], cigarette smoking [14], calcium intake [11,12,15], history of contraceptives [16] and level of physical activity [11,17,18] (Table 1). The women were asked specifically whether they were still menstruating or not. We then used the WHO definition that requires 12 months of continuous amenorrhoea [19] to define menopause and, counting one year backwards from baseline, set the threshold for early menopause at age 47. BMD, one of the strongest recognised predictors of fracture [1,20], was measured by single-photon absorptiometry (SPA), which gauges forearm BMD at a site 6 cm proximal to the styloid process of the ulna, as described by Nauclér et al. [21]. Greater height [22,23], lower body weight [24,25] and low body mass index (BMI) [11,26] are all reported risk factors for fracture and these traits were measured by standard equipment. Body mass index (BMI) was calculated as body weight divided by body height squared (kg/m^2).

Although the study was not originally designed to identify risk factors for higher mortality, several of the baseline variables have also been associated with increased mortality risk, such as early menopause [5], smoking [27], excess body weight [28,29], low body weight [29], low BMD [30] and low physical activity [31].

During the follow-up period of 34 years we registered and classified incident fragility fractures using a thorough and well validated system, as described in detail in our previous report [3]. We followed the women until death, relocation or until the end-point date 30 September 2011 using the hospital registers and digitised databases or in nine cases, through telephone interviews. All fractures were verified through case reports and our fracture records were complete in all but 11 cases; these women had either died after having relocated or could not be located. Mortality data were obtained from the national population records, which do not register cause of death. For the fracture evaluation, the mean follow-up time was 25.9 years and the median was 30.7 years (range 0.9, 34.0). For the mortality evaluation, the mean follow-up time was 30.1 and the median was 33.6 years (range 0.8, 34.0).

The study was approved by the Ethics Committee of Lund University and conducted in accordance with the norms of the Helsinki Declaration of 2001. Written informed consent was obtained from each individual. The technical equipment was validated by the Swedish Radiation Protection Inspectorate and by the hospital's own radiation protection committee. The Swedish Data Inspection Board approved both the data collection and the database. Statistical analyses were performed using STATISTICA software version 7.1 (StatSoft). Data are shown as means with 95% confidence intervals (95% CI). Group comparisons of baseline variables when comparing women who were to sustain a fragility fracture with those who were not and women who later died and those who did not, were made with chi squared tests and the Student's *t*-test between means. Univariate survival analysis was performed with Cox's proportional hazard regression, calculating a risk ratio

Table 1
Baseline characteristics in the 390 women aged 48 at study start, dichotomised into groups according to whether they were to sustain a fragility fracture during follow-up or not, and whether they died or stayed alive during the follow-up period.

Variable		Fracture cohort (n = 128) Mean (95% CI)	Non-fracture cohort (n = 262) Mean (95% CI)	p-Values	Mortality cohort (n = 148) Mean (95% CI)	Non-mortality cohort (n = 242) Mean (95% CI)	p-Values
Age	(Years)	48.3 (48.2, 48.3)	48.3 (48.3, 48.3)	0.22	48.3 (48.3, 48.3)	48.3 (48.3, 48.3)	0.18
Menarche	(Years)	14.1 (13.8, 14.3)	14.0 (13.8, 14.1)	0.55	14.0 (13.8, 14.3)	14.0 (13.8, 14.2)	0.78
Height	(cm)	163.8 (162.8, 164.7)	164.1 (163.4, 164.8)	0.57	164.1 (163.2, 165.0)	164.0 (163.3, 164.6)	0.80
Weight	(kg)	62.0 (60.4, 63.6)	64.2 (63.0, 65.4)	0.03	64.0 (62.2, 65.7)	63.2 (62.0, 64.3)	0.43
Forearm BMD	(g/cm^2)	525 (514, 535)	549 (541, 556)	<0.001	540 (530, 551)	541 (534, 549)	0.92
Forearm bone width	(mm)	13.5 (13.2, 13.7)	13.5 (13.3, 13.6)	1.00	13.4 (13.2, 13.6)	13.5 (13.3, 13.7)	0.45
		Number (%)			Number (%)		
Menopausal status	Yes	27 (21%)	34 (13%)	0.07	32 (22%)	29 (12%)	0.01
	No	101 (79%)	228 (87%)		116 (78%)	213 (88%)	
History of breast feeding	Yes	103 (80%)	202 (77%)	0.51	118 (80%)	187 (77%)	0.43
	No	22 (17%)	52 (20%)		25 (17%)	49 (20%)	
	Missing data	3 (2%)	8 (3%)			6 (2%)	
Children	0	15 (12%)	37 (14%)	0.79	20 (14%)	32 (13%)	0.10
	1–3	104 (81%)	207 (79%)		113 (76%)	198 (82%)	
	>3	9 (7%)	17 (6%)		15 (10%)	11 (5%)	
	Missing data	0	1				
Current physical activity	High	30 (23%)	87 (33%)	<0.05	42 (22%)	75 (31%)	0.58
	Low	98 (77%)	175 (67%)		106 (78%)	167 (69%)	
Current smoking	Yes	62 (45%)	122 (47%)	0.60	46 (31%)	97 (40%)	0.19
	No	57 (48%)	126 (48%)		87 (59%)	137 (57%)	
	Missing data	9 (7%)	14 (5%)		15 (10%)	8 (3%)	
History of oral contraceptives	Yes	31 (24%)	73 (28%)	0.45	41 (28%)	63 (26%)	0.72
	No	97 (76%)	189 (72%)		107 (72%)	179 (74%)	
Current calcium intake	<400 mg/day	25 (20%)	46 (18%)	0.62	26 (18%)	45 (19%)	0.77
	≥400 mg/day	102 (80%)	215 (82%)		122 (82%)	195 (80%)	
	Missing data	1	1			2 (1%)	

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Table 2
Univariate analyses of risk ratios (RR) with 95% confidence intervals for fragility fracture and mortality, analysed with Cox's proportional hazard regression, and with significant risk ratios in extra bold type.

Variable	Risk ratio for fragility fracture 2011	*p*-Value	Mortality risk ratio 2011	*p*-Value
Age at menarche (per SD decrease)	0.97 (0.81, 1.15)	0.71	0.95 (0.81, 1.12)	0.56
Height (per SD decrease)	1.02 (0.85, 1.22)	0.82	0.96 (0.82, 1.14)	0.66
Body weight (per SD decrease)	1.19 (0.98, 1.43)	0.07	0.92 (0.78, 1.08)[a]	0.31
Body Mass Index (per SD decrease)	1.19 (0.98, 1.44)	0.08	0.93 (0.79, 1.09)[b]	0.39
Forearm BMD (per SD decrease)	1.40 (1.18, 1.67)	<0.001	1.01 (0.85, 1.19)	0.94
Forearm bone width (per 1SD decrease)	1.02 (0.85, 1.22)	0.83	1.05 (0.90, 1.24)	0.52
Strength index (per SD decrease)	1.19 (0.98, 1.44)	0.07	1.05 (0.89, 1.24)	0.56
Menopausal (vs non-menopausal)	1.76 (1.15, 2.70)	<0.01	1.72 (1.16, 2.54)	0.01
History of breast feeding (vs no breast feeding)	1.18 (0.75, 1.88)	0.47	1.18 (0.76, 1.81)	0.46
Number of children[c]	1.17 (0.78, 1.75)	0.46	1.26 (0.87, 1.84)	0.24
Low physical activity (vs high physical activity)	1.47 (0.98, 2.22)	0.06	1.12 (0.79, 0.61)	0.52
Current smoking (vs no smoking)	1.22 (0.85, 1.75)	0.28	2.30 (1.61, 3.29)	<0.001
History of oral contraceptives (vs no history of intake)	1.09 (0.73, 1.63)	0.68	0.93 (0.65, 1.33)	0.69
Low calcium intake (vs high intake)	1.19 (0.77, 1.34)	0.45	0.95 (0.62, 1.44)	0.79

[a] Inverted RR for mortality per SD increase BMI was 1.09 (0.93, 1.27).
[b] Inverted RR for mortality per SD increase body weight was 1.07 (0.91, 1.26).
[c] Variable grouped into 0, 1–3 or >3 children, calculated as a continuous variable with RR for each step.

(RR) with a standard deviation for every variable. Variables with $p < 0.20$ were then included in a multivariate analysis to estimate whether the identified risk factor remained an independent predictor. If there was a correlation of $r \geq 0.40$ between two identified risk factors, only one of the variables was included in the multivariate analysis.

3. Results

Table 1 shows baseline characteristics at age 48 in women who were to sustain a fragility fracture and those who were not and women who later died and those who did not. Women who were to have a fracture had lower BMD at baseline ($p < 0.001$), lower body weight ($p < 0.05$) and a lower level of physical activity ($p < 0.05$) than women who were not to sustain fractures (Table 1). In the women who later died, more were menopausal at baseline than in those who stayed alive ($p = 0.01$) (Table 1).

Fracture risk and mortality risk during the 34-year follow-up period for the baseline variables (Table 1) were calculated in a univariate analysis. These analyses found that early menopause ($p < 0.01$) and low BMD ($p < 0.001$) were associated with fractures, while the higher risk in women with low body weight ($p = 0.07$), low BMI ($p = 0.08$) and current low physical activity ($p = 0.06$) did not reach statistical significance (Table 2). Risk factors with a *p*-value of <0.2 in the univariate analyses were adjusted for each other in a multivariate analysis which showed that only low BMD remained an independent predictor with a RR of 1.36 (95%CI 1.14, 1.62) per SD decrease in baseline BMD, whereas early menopause now reached only borderline significance with a RR of 1.49 (95%CI 0.97, 2.29) (Table 3).

In the univariate analyses, early menopause ($p = 0.02$) and current smoking ($p < 0.001$) were associated with mortality, and these variables remained independent risk factors in the multivariate analysis, now with RR of 1.62 of (95%CI 1.09, 2.39) and 2.16 (95%CI 1.53, 3.06), respectively (Table 3).

4. Discussion

The results of this study support the notion that early menopause is associated with higher risk of fragility fracture [4] and mortality [3,5]. We suggest that the ability to predict fracture is mediated by other risk factors associated with early menopause, chiefly low BMD. In contrast, we found that early menopause was an independent risk factor for mortality, indicating that the inferred mortality risk is associated with risk factors other than those identified in this survey. Furthermore, this study corroborates the standard beliefs that smoking has a strong influence on mortality risk [27] and that low BMD is an independent predictor of fracture [1,20].

While the majority of published reports concur that early menopause predicts low BMD and fragility fractures in the close post-menopausal period [4], the association has been less obvious in higher ages [6–8]. The current study indicates that the impact of early menopause prevails in the remote post-menopausal period, with an increased fracture and mortality risk. The association of early menopause with increased risk of fracture and osteoporosis is mainly documented in cross-sectional or prospective short-term studies [4], but is also supported by our group in a previously published prospective long-term observational report [3]. However, in this study we only evaluated early menopause as a predictor, not the association with other risk factors.

The grounds for the association between early menopause and a higher fracture risk can only be speculative, since we cannot prove causality in an observational study. It is conceivable that an early menopausal transition and the consequently altered hormonal environment leads to a deteriorated health status and a subsequent higher fracture risk; in other words, that fracture is predicted by early menopause as mediated by other risk factors. One of the most widely recognised risk factors for fractures is low BMD [1,20]. Early menopause is evidently associated with low BMD [4] and it has been established in prospective epidemiological studies that a 1 SD decrease in BMD implies twice the risk of fracture

Table 3
Multivariate analysis with Cox's proportional hazard regression, including risk factors with $p < 0.20$. Body weight was used instead of BMI since these variables correlated *r* 0.90.

	RR for fracture	*p*-Value		RR for mortality	*p*-Value
Forearm BMD	1.36 (1.15, 1.62)	<0.001	Early menopause	1.62 (1.09, 2.39)	0.02
Early menopause	1.49 (0.97, 2.29)	0.07	Smoking	2.16 (1.53, 3.06)	<0.001
Body weight	1.16 (0.96, 1.40)	0.12			
Physical activity	1.36 (0.90, 2.06)	0.14			

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[20,32]. The multivariate analyses in this study also indicate that low BMD is responsible for a large proportion of the association between early menopause and fragility fractures. However, other factors that were not measured in our study, such as inferior muscle strength or a reduced neuromuscular function, may also partially explain the relationship. Furthermore, in this study only predictors at age 48 were analysed (these variables could alter during follow-up) and secondly, we cannot exclude the possibility that there were clinically important differences in the baseline variables that we could not capture by our questionnaire. In addition, other risk factors for falls, such as morbidity and medication, might appear only after study start and thus escape being identified as risk factors. Finally, another explanation model is that one or more genetic variants or other unidentified confounding factors present already at study start could be the common grounds for both early menopause and the higher fracture incidence. Early menopause would then be a marker of risk but not a causal factor.

The same considerations apply to the high mortality risk in women with early menopause. Our observational study design does not allow inferences on causal relationships. Our result is, however, in accordance with the conclusions of Shuster et al. [5] who found that menopause before the age of 45 was associated with an increased risk of overall mortality. As discussed by the authors, early menopause may be the result of generally impaired health and not the causal factor. Inversely, the menopausal transition itself could also play a causal role in the increased mortality risk through detrimental physiological effects mediated by hormonal mechanisms. Regardless of the chain of events, however, early menopause appears to be an indicator of a premature ageing process and thus also a predictor of fragility fracture and mortality.

Our study was not originally designed to evaluate risk factors for mortality. The baseline variables were selected for their association with bone mass and fractures although early menopause [5], smoking [27], excess or low body weight [28,29], low BMD [30] and physical activity [31] have all been associated with increased mortality risk, too. Several commonly recognised risk factors for mortality were not evaluated in this study and therefore it is not surprising that the significantly higher mortality risk in women with early menopause remained after adjustments. Our interpretation is that the increased mortality risk could be mediated by risk factors that are associated with early menopause but not included in this study.

We also found that smoking was an independent risk factor for mortality, not surprisingly since its close association with pulmonary and cardio-vascular disease is well documented [27]. Interestingly, early menopause has been strongly associated with smoking [33–35] and also with low body weight [35,36], which reached an almost significant risk ratio for fractures in our study. Once more, these variables could be part of complex underlying circumstances that this study was not designed to explore.

It should also be underlined that published results are not unanimous regarding the long-term influence of early menopause on fracture risk. Van der Voort et al. [37] found an increasing predictability of fracture by early menopause after age 70, whereas other studies have indicated a fading influence on BMD [6,8] and fracture risk [7] with increasing age. This is probably due to the increasing number of other factors that are influencing bone mass and fracture risk in ageing women, and gradually diminishing the effects of early menopause. In this perspective, it is interesting to discuss why our results differ. This may be a methodological issue, given the homogeneous population in our study as regards age, ethnicity and location. The women in our cohort were followed from the same chronological baseline in 1977 and the vast majority lived in the same city all through the study period and was therefore exposed to the similar environmental factors during their ageing. In addition, there was little variation in the baseline variables between groups, meaning that the difference in menopausal status at baseline would gain a greater impact.

The strengths of this study include the population-based long-term prospective study design with a homogeneous population, the 97% participation rate in the fracture and mortality evaluation and the unprecedented study length. The fracture registration through a well-validated system that only includes objectively verified fractures must also be regarded as a study strength. The definition of menopause using the WHO classification [19] and the classification of menopause as a dichotomous variable at baseline, instead of using retrospectively estimated menopausal age as in most cited studies, must also be regarded as advantageous. Study limitations include the fixed definition of early menopause; it would have been interesting to evaluate an even lower cut-off age for early menopause, since published studies suggest that the earlier the onset, the longer the impact [4]. However, this would have had to be based on retrospective estimations of age at menopause, with the risk of recall bias. It should also be acknowledged that there are important risk factors for fracture and mortality that were not evaluated in this study. Data on cause of death, morbidity, medications and changes in lifestyle during the follow-up period would have been valuable to include. Particularly, complete records of hormone replacement therapy and bisphosphonate use would have been of interest, given the effect on fracture reduction and mortality risk associated with these medications [38–40]. We cannot exclude the possibility that our results could have been different had we been able to control for these factors.

With the restrictions discussed above in mind, we argue that menopause before the age of 47 could be a useful and easily accessible tool for prediction of fracture and mortality. It remains unclear whether early menopause is a causal factor or the result of unidentified background mechanisms present already at menopause, leading to both more fractures and mortality and an early menopause. Low BMD stands out as the principal independent risk factor for fracture in the postmenopausal period.

Conflicts of interest statement

All authors state that they have no conflicts of interest.

Contributors

I declare that I participated in designing the study, the calculation of the data and was the main author of the text, and that I have seen and approved the final version.

I have no conflicts of interest.

Ola Svejme, main author

I declare that I participated in the planning and designing of this study, the data collection, and that I have seen and approved the final version.

I have no conflicts of interest.

Henrik G. Ahlborg, co-author

I declare that I participated in designing the study and analysing the data, that I was the co-author of the text, and that I have seen and approved the final version.

I have no conflicts of interest.

Magnus K. Karlsson, co-author

I declare that I participated in the statistical analysis of the data and that I have seen and approved the final version.

I have no conflicts of interest.

Jan-Åke Nilsson, statistician

I declare that I performed the language revision of this manuscript and that I have seen and approved the final version. I have no conflicts of interest.

Adam Crozier, language consultant

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Competing interest

None

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Ethics approval

The study was approved by the Lund University Ethics Committee.

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